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EFFECTS OF RADIOACTIVE PHOSPHORUS (P^{32}) ON NORMAL TISSUES

A Histologic Study of the Changes Induced in the Organs of Patients with
Malignant Lymphomas

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ST. LOUIS

RADIOPHOSPHORUS has been used for the last seven years in the experimental and clinical study of various blood dyscrasias, especially primary polycythemia and the leukemias. Although much has been written concerning the distribution and deposition of this radioactive element in the peripheral blood and body tissues of certain laboratory animals and individual patients, few reports¹ have been made on the histologic changes induced by this type of beta particle radiation. Warren and Cowing^{1a} have studied the effects of the intraperitoneal inoculation of radioactive phosphorus on the liver, voluntary muscle, bone, spleen and kidneys in rats. This scarcity of articles on the histologic effects of radiophosphorus is probably related to the fact that the biologic effects of radiophosphorus are qualitatively similar to those of roentgen radiation—the effects of roentgen rays on normal tissues having been fully described by Warren and his co-workers.² Although most of the pathologic changes produced by this temporarily radioactive isotope (P^{32}) are identical with those seen after the application of roentgen radiation, there are certain differences in the mode of administration, the duration of activity and the selective concentration which may serve to explain certain tissue alterations produced by it.

PREPARATION AND USAGE OF RADIOACTIVE PHOSPHORUS

The radiophosphorus used in the patients studied in this series³ was prepared in the cyclotron of the Mallinckrodt Institute of Radiology by the bombardment of red phosphorus with 12,000,000 volt deuterons (nuclei of the heavy isotope of hydrogen, called deuterium): In this procedure, ordinary stable red

From the Department of Pathology, Washington University School of Medicine.

1. (a) Warren, S., and Cowing, R. F., abstracted, Arch. Path. **29**:730, 1940.
(b) Warren, S., and Gates, O.: *ibid.* **30**:440, 1940.

2. (a) Warren, S.: Arch. Path. **34**:443, 1942. (b) Dunlap, C. E., and Warren, S.: *ibid.* **34**:562, 1942.

3. Reinhard, E. H.; Moore, C. V.; Bierbaum, O. S.; Moore, S., and Kamen, M. D.: J. Lab. & Clin. Med. **31**:107, 1946.

phosphorus, previously smeared on a copper target, is placed directly in the path of a beam of high speed deuterons emitted by a cyclotron. The nuclei of a small fraction of the phosphorus atoms then receive a neutron each as a result of this bombardment. Ordinary stable phosphorus has 15 protons and 16 neutrons, each having a mass of 1—making an atomic mass of 31 for this element. Ordinarily the mass of any atom is written to the right and slightly above the chemical symbol of the atom. Therefore, stable phosphorus is referred to as P^{31} . The atomic mass is increased by 1 when a neutron enters the phosphorus nucleus, and the resultant nucleus has a mass of 32. Radioactive phosphorus is, therefore, referred to as P^{32} ; its nucleus is unstable and the atom radioactive. The effect of radioactive phosphorus on tissues is exerted when an electron or beta particle is emitted from the substance. When this occurs, radiophosphorus is transformed into ordinary stable sulfur; i. e., when one of the neutrons in the nucleus changes into a proton, the positive charge on the nucleus is increased by 1 and a new nucleus is formed (sulfur).

The phosphorus, prepared as described, is then synthesized into its dibasic sodium salt,⁴ and sufficient distilled water is added to make an isotonic solution (15 mg. Na_2HPO_4 per cubic centimeter). A Lauritsen electroscope (calibrated with a uranium standard) is used to determine the radioactivity of the phosphate solution. The freshly prepared solutions usually showed activity varying between 0.20 and 0.40 millicurie per cubic centimeter. (A millicurie is that amount of any radioactive substance in which 37,000,000 atoms disintegrate per second.⁵ Therefore, a microcurie is the amount of any radioactive substance which disintegrates at the rate of 37,000 atoms per second. If 1.1 microcuries of radiophosphorus completely disintegrates inside of a kilogram of tissue, it will deliver to that tissue 1 roentgen equivalent of radiation.) Each dose of radioactive phosphorus, which was given parenterally, varied between 0.1 and 2.5 millicuries (100 and 2,500 microcuries). At first, patients were usually treated two or three times a week. The changes observed in the peripheral blood served to control the therapy. In cases of leukemia the cellular components of the blood were restored to as nearly a normal picture as was possible and an attempt was made to maintain this effect. In the other cases (lymphosarcoma, multiple myeloma, Hodgkin's disease and other diseases), in which there was no elevation of the white blood cell count, radioactive phosphorus was given until peripheral blood changes denoted depression of activity of the bone marrow.

HISTOLOGIC OBSERVATIONS

In this series of 43 cases in which tissue changes produced by radiophosphorus were studied microscopically, the diagnoses were as follows: acute leukemia, 4; chronic leukemia, 21; leukosarcoma, 9; aleukemic leukemia, 1; Hodgkin's disease (lymphogranulomatosis), 2; multiple myeloma, 3; lymphosarcoma, 1; melanoma, 1; and Ewing's sarcoma (angioendothelioma of bone), 1. The ages of the patients with leukemia, who constituted the largest group, varied between 9 and 65 years. Patients with malignant lymphomas who had not been treated with any type of radiation were studied as controls. The gross changes attributable to radiophosphorus observed in various organs were minimal when compared with the alterations demonstrated microscopically throughout most of the tissues examined. The accompanying table shows the radioactivity of the tissues studied in 32

4. Hempelmann, C. H., Jr.; Reinhard, E. H.; Moore, C. V.; Bierbaum, O. S., and Moore, S.: *J. Lab. & Clin. Med.* 29:1020, 1944.

5. Low-Beer, B. V. A.; Lawrence, J. H., and Stone, R. S.: *Radiology* 39:573, 1942.

Patient	Total Dose in Millieuries	Time in Days Total Dura- from tion of Last Treat- Injec- ment in tion Weeks of P-32	Bone Marrow †	Bone	Liver	Spleen	Kidney	Muscle	Lymph Node	Brain	Lung	Other Organs
A10915	19,703	74	6	0.064 (1)	0.043 (4)	Myelogenous Leukemia 0.032 (5)	0.048 (3)	0.023 (6)	0.049 (2)			
A11275	69,863	26	60	0.0232 (2)	0.0218 (3)	0.0148 (4)	0.0113 (5)	0.0079 (6)	0.0238 (1)			
A1424J	44,957	52	1	0.294 (1)	0.218 (3)	0.232 (2)	0.152 (4)	0.098 (5)		0.021		0.040 (blood)
A11217	44,236	53	7	0.144 (1)	0.132 (2)	0.128 (3)	0.080 (3)	0.032 (5)				
A11195	18,317	22	12	0.0473 (1)	0.0470 (2)	0.0279 (4)	0.0305 (3)	0.0216 (6)	0.0278 (5)			0.0059 (blood)
A10939	4,832	1	3	0.263 (1)	0.088 (2)	0.081 (3)	0.051 (5)	0.020 (6)	0.063 (4)			
A11179	13,956	11	26	0.0295 (1)	0.0274 (2)	0.0170 (5)	0.0139 (6)	0.0178 (4)	0.0243 (3)			
A11022	21,558	50	10	0.0695 (1)	0.0267 (3)	0.0236 (3)	0.0145 (4)	0.00995 (5)		0.0025		
A11506	19,518	31	33	0.00527 (1)	0.00463 (2)	0.00355 (3)	0.00275 (4)	0.00237 (5)		0.0021		
A11215	3,470	1/2	1	0.0523 (1)	0.0857 (2)	0.0650 (3)	0.560 (4)	0.0308 (5)			0.0665	
A11638	31,440	147	11	0.00905 (2)	0.0103 (1)	0.00624 (5)	0.00681 (4)	0.00482 (6)	0.00886 (3)	0.00662		
A1494J	8,446	5	5	0.034 (3)	0.101 (1)	0.071 (2)	0.059 (3)	0.043 (4)			0.055	
A11495	23,649	31	21	0.437 (3)	0.0535 (2)	Lost	0.0342 (4)	0.0270 (4)	0.0547 (1)			
A11476	9,364	7	1	0.0384 (4)	0.173 (1)	0.152 (2)	0.0886 (5)	0.0417 (6)	0.135 (3)	0.0184		
A10808	25,800	26	8	0.0297 (4)	0.0483 (2)	Leukosarcoma 0.0504 (1)	0.0408 (3)	0.0249 (5)		0.0120		0.0207 (intestine)
A11788	35,619	103	5	0.0578 (1)	0.0515 (3)	0.0325 (2)	0.0371 (4)	0.0141 (6)	0.0338 (5)			
A10754	4,630	3	4	0.0366 (3)	0.0982 (1)	0.0534 (2)		0.0237 (4)	0.0193 (5)	0.0120		
A11063	13,205	70	1	0.145 (1)	0.1097 (2)	0.0664 (3)	0.0498 (4)	0.0639 (5)				
A11207	17,250	29	8	0.165 (3)	0.203 (1)	0.180 (2)	0.136 (3)	0.091 (5)		0.050		
A11089	10,088	4	2	0.310 (3)	0.375 (1)	0.350 (2)	0.280 (4)	0.0856 (6)	0.180 (5)			0.0864 (blood)
A11124	6,858	4	3	0.0504 (4)	0.0827 (1)	0.0636 (3)	0.0654 (2)	0.0406 (6)	0.0420 (5)			0.01625 (blood)
A11494	9,560	6	7	0.0520 (3)	0.0570 (1)	0.0443 (4)	0.0434 (5)	0.0190 (6)	0.0559 (2)		0.01375	
A11045	5,210	2	2	0.120 (2)	0.124 (1)	Monocytic Leukemia 0.102 (4)	0.090 (5)	0.043 (6)	0.116 (3)			0.103 (blood)
A11408	10,136	8	10	0.0915 (1)	0.083 (3)	0.086 (2)	0.0615 (4)	0.0392 (6)	0.0556 (5)			
A11730	4,964	1	4	0.0620 (2)	0.0535 (3)	0.0640 (1)	0.0332 (5)	0.0263 (6)	0.0474 (4)			
A11570	4,504	1/2	1	0.0590 (2)	0.104 (1)	0.0727 (4)	0.0817 (3)	0.0426 (5)				
A10797	15,081	18	10	0.0190 (4)	0.0501 (1)	Reticulum Cell Sarcoma 0.0422 (2)	0.0357 (3)	0.0077 (5)			0.0211	0.0603 (tumormass)
A10568	11,993	31	24		Hodgkin's Disease (Lymphogranulomatosis) 0.0444	0.008						
A10698	8,000	7	1	0.1688 (4)	0.3442 (1)	Multiple Myeloma 0.1949 (2)	0.1846 (3)	0.0656 (5)				
A110645	5,002	2	4		0.1020 (1)	0.0660 (2)	0.0590 (3)			0.0087		0.1087 (tumor), 0.0310 (intestine)
A11326	10,908	16	61	0.00268 (1)	0.00109 (3)	0.00119 (2)	0.000754 (4)	0.000745 (5)				
A11580	7,748	4	5	0.138 (1)	0.0840 (3)	Malignant Melanoma 0.0794 (4)	0.0517 (5)	0.0194 (6)	0.0966 (2)		0.0615	0.114 (tumor)

* This table is reprinted with modifications by permission of Drs. E. Reinhard and Carl V. Moore from the Journal of Laboratory and Clinical Medicine (31: 118, 1946).

† The radioactivity is expressed in microcuries per gram of wet tissue, with the relative activity of the various tissues represented by numbers in parentheses. The rating 1 indicates the least activity and the rating 6 the greatest activity.

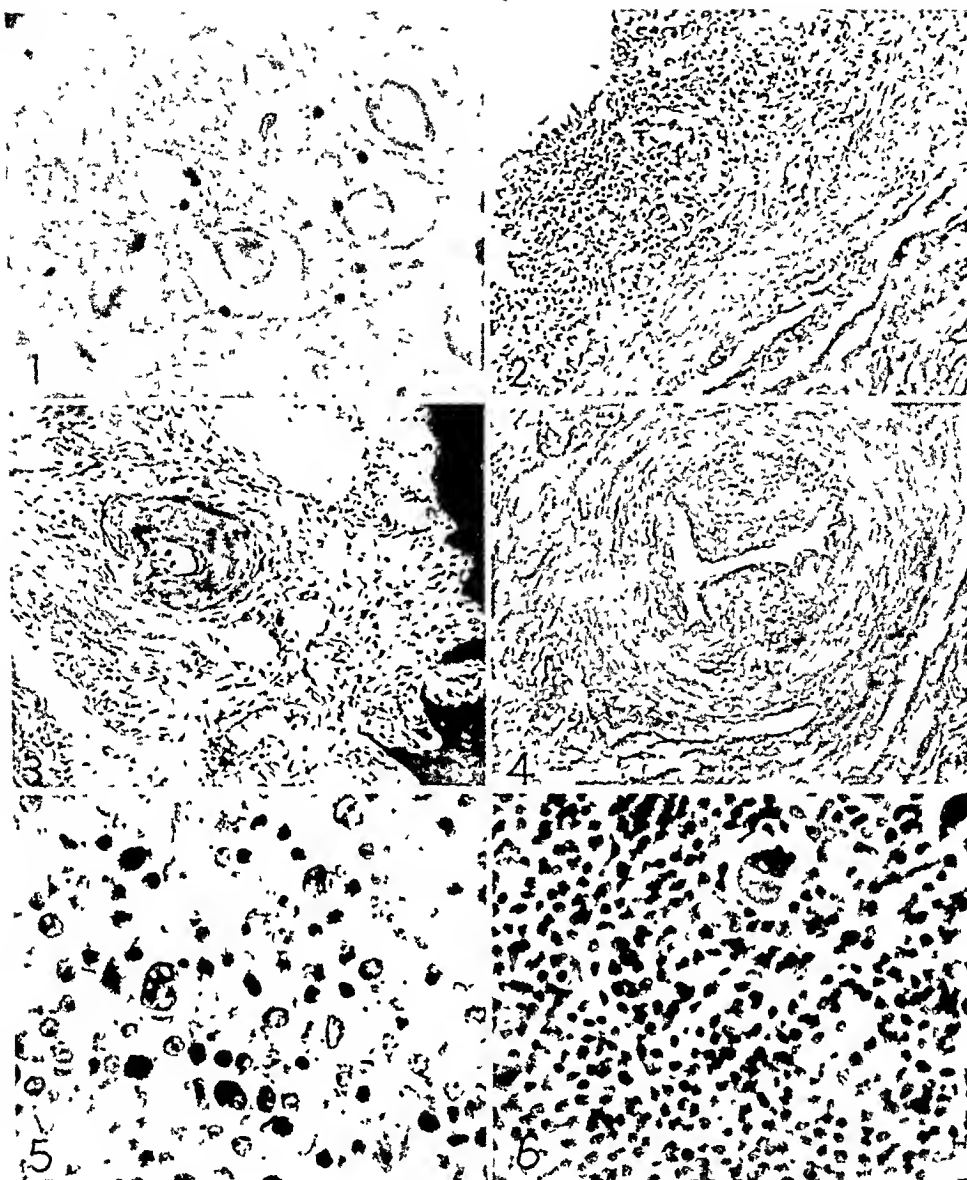


Fig. 1.—Medulla oblongata. Note the eosinophilic outline of neurons showing disappearance of Nissl granules and eccentric location of the pyknotic nuclear remnant. Hematoxylin and eosin stain; $\times 340$.

Fig. 2.—Colon showing atrophy of acini and desquamation of the mucosal epithelium. There is marked hyalinized fibrosis of the mucosa and submucosa, with telangiectasis of vessels in the submucosal layer. Hematoxylin and eosin stain; $\times 79$.

Fig. 3.—Vertebral marrow showing replacement of normal cellular elements by a loose edematous connective tissue containing dilated capillaries. There is marked hyalinization of the medial coat of the artery, with narrowing of its lumen. Hematoxylin and eosin stain; $\times 79$.

Fig. 4.—Uterine artery of 28 year old white woman (no previous pregnancies). The marked proliferation and swelling of the endothelial cells produce thickening of the intima and narrowing of the lumen. Elastic and muscle fibers in the media show fraying and separation with increase in the number of collagen fibers. Hematoxylin and eosin stain; $\times 79$.

Fig. 5.—Lymph node showing large multinucleated atypical giant cells with fused pyknotic nuclei and a rim of basophilic cytoplasm. Hematoxylin and eosin stain; $\times 340$.

Fig. 6.—Lymph node in Hodgkin's disease. Compare the Reed-Sternberg cell with the cells in figure 5 showing multiple, fused, pyknotic nuclei and dark eosinophilic cytoplasm. Hematoxylin and eosin stain; $\times 340$.

of the 43 cases, with the relative activity of the various tissues represented by numbers in parentheses placed to the right of the activity in microcuries. Bone marrow, liver, spleen, kidney, muscle and lymph node were the only six tissues considered in the determination of these relative activities. The rating 1 indicates the greatest activity, whereas the rating 6 indicates the least activity. This distribution of radioactivity was determined by accurately weighing 1 Gm. samples of the fresh tissues removed at autopsy. Digestion with concentrated sulfuric acid and 30 per cent hydrogen dioxide was then individually performed in a Kjeldahl flask. After the clear digest had been diluted with distilled water to a volume of 50 cc., various aliquots of this solution were measured into the glass well of a plunger or a dipping type Geiger counter (Bale tube) until a dilution was found which gave approximately the same number of counts per minute as did a standard radiophosphorus solution of known activity. The radioactivity in microcuries of wet tissue was then calculated from this determination.³

Brain.—Permission for an examination of the cerebral tissues was granted in 11 cases, in 8 of which there was a diagnosis of leukemia. In the remaining 3 no metastatic or infiltrative lesions were shown. Hemorrhage and leukemic cell infiltration of the brain, occurring separately or together, were the only significant pathologic changes that could be directly related to the primary disease. However, the nerve cells in the cortex and the white matter situated at a distance from the leukemic alterations showed definite retrogressive changes in a few cases. These consisted of complete disappearance of Nissl granules, loss of nuclei, pyknosis of chromatin matter, swelling and chromatolysis. In some instances mere eosinophilic outlines of former neurons remained (fig. 1). The cerebellum revealed only mild degenerative changes, such as breaking up of cell membranes, loss of Nissl bodies and sometimes disappearance of nuclei. Occasionally foci of neuroglial cell increase were seen, especially in the regions of the cerebrum where the nerve cells were undergoing degeneration. Other changes infrequently noted were perivascular lymphocytic infiltration and meningeal arteriolar hypertrophy with hyalinization of the media and swelling of the intimal coat. In some cases the choroid plexus showed thickening of the villi by an increase of connective tissue in their central cores. The small blood vessels and capillaries in these regions appeared to be somewhat dilated; others were completely obliterated by the sclerotic process. All the preceding changes of glia and stroma were only occasionally observed in the central nervous system; hence it may be concluded that radioactive phosphorus has little effect on nerve tissue. No clinical neurologic observations could be directly or indirectly attributed to these minimal microscopic changes. More than likely, the vascular damage with the associated focal ischemia, rather than direct injury of the nerve elements, is the mechanism of injury in the brain when radiation is applied by isotopic therapy.

Skin.—The epidermis and dermis exhibited pathologic changes which were quite similar to those seen following direct irradiation.⁶ Most of the sections examined showed changes ranging from extreme atrophy, disarrangement and disappearance of the basement membrane of epidermal cells to marked hyperkeratosis. There was leveling of the rete pegs, with the cells of the stratum germinativum resting on a hyalinized corium, which contained dense collagen with few elastic fibers. No ulceration was seen. The accessory appendages were atrophic, degenerative and even missing in most of the sections of skin. Only occasional areas showed dilatation of capillaries, arterioles, venules and lymphatics. None of the extreme telangiectatic changes as observed in cases in which erythema and dermatitis were associated with local application of roentgen rays could be demonstrated in these cases of radiophosphorus treatment.

Esophagus.—The stratified squamous epithelium covering the mucous membrane of the esophagus reveals the same type of response that was seen in other epithelium-lined internal structures; that is, vacuolation and desquamation of the epithelial cells with edema of the submucosa and infiltrating abnormal blood cells. Fibrosis and thickening of the esophageal musculature were also seen in some instances. In the distal part of the esophagus there were moderate vacuolation of the cells lining the cardiac glands and hypersecretion of mucus. In the evaluation of the aforementioned observations it is necessary to bear in mind that foci of necrosis and subsequent ulceration with its associated chronic inflammatory reaction are frequently found in leukemia, especially in those patients with a low granulocyte count and infection. However, since patients given radiophosphorus treatment present depression of the leukopoietic function of the marrow (see description of bone marrow), with subsequent agranulocytosis and terminal oropharyngeal-esophageal ulceration observed more frequently than in the series of control untreated patients, these conditions may perhaps be caused by the radioactive phosphorus.

Gastrointestinal Tract.—Examination of the gastrointestinal tissues in the 43 cases in this series revealed in 25 the following distribution of lesions which could be attributed to beta ray effect: colon, 9; appendix, 6; small intestines, 6; stomach, 4. Grossly, the intestinal wall was only occasionally thickened and indurated; the serosa was rarely opaque, with moderate telangiectasis. The mucosal epithelial lining showed occasional foci of superficial ulceration (not associated with neoplastic cell infiltration). Regenerating flat to cuboidal epithelium seemed to grow in from the edge of the ulcer, while remnants of the glands within the eroded area sometimes became hyperplastic, with extremely prominent mucous type cells. Those areas of the mucosa which were not ulcerated showed diffuse atrophic changes (fig. 2). Overproduction of mucus by goblet cells, which were enlarged and increased in number, was occasionally seen in distorted glands. The latter were frequently narrowed and elongated or dilated and cystic and were often lined by flattened epithelium. Swollen nuclei with prominent nucleoli were also observed. Occasionally, multinucleated, bizarre, atypical giant cells were seen between the acini and in the submucosal coat proper. Usually accompanying these alterations there was moderate dilatation of lymphatics, capillaries and veins in the mucosa and submucosa. The muscle fibers were the sites of hyaline degeneration, interstitial fibrosis, edema, vacuolation and atrophy.

Liver.—The changes in this organ which could be directly attributed to radiophosphorus were minimal when compared with the more frequently observed alterations in hepatic cells resulting from the anoxia of the severe associated anemia and with the degenerative changes secondary to the leukemic cellular infiltration. Perhaps the most outstanding observation was the variation in size and in chromatism of the hepatic nuclei. There was also fusion of adjacent nuclear structures. The Kupffer cells seemed to enlarge and assumed irregular shapes. There were occasional foci of necrosis, in various stages of degeneration, in the pericentral, midzonal and periportal areas of the hepatic lobule. Some involved the greater part of the lobule and were severe. It is difficult to state specifically whether these changes were secondary to the anoxemia which so frequently accompanies these diseases or whether they were due to the effects of radiophosphorus. There also seemed to be an increased amount of fibrous tissue in the capsule and in the portal regions, with dilatation and slight endophlebitis of some of the radicles of the portal vein. Sclerotic changes

involving the hepatic arteries and proliferative hypertrophy and hyperplasia of the biliary epithelium were minimal.

Bone Marrow.—Sections of vertebral, sternal, rib and femoral marrow were examined. Myeloid hyperplasia was shown in 20 cases; lymphoid hyperplasia and infiltration, with hypoplasia of the other formed elements, in 2; hypoplasia of all the blood cells in 2, and diffuse necrosis and fibrosis of the medullary cavities of both flat and long bones in 18. Macroscopically, the marrow was of a gray to grayish red color and was somewhat less porous than the untreated leukemic or the normal marrow. The histologic changes in the marrow were not completely reflected in the circulating blood. Distributed throughout the hyperplastic marrow there were occasional deposits of hemosiderin, foci of hemorrhage, foci of cell-poor gelatinous edema and regions of fibrosis of varying density, size and maturity. The hypoplastic to aplastic marrows revealed replacement of the normal cellular elements by a loose edematous connective tissue or by a hyalinized collagenous material in which scattered islands of hemopoiesis and rare megakaryocytes survived (fig. 3). In 11 cases large atypical giant cells were observed, many multinuclear and an occasional uninuclear cell.

Lymph Nodes.—In considering the histologic changes observed in lymphoid tissue after the administration of radiophosphorus the cases of Hodgkin's disease, lymphosarcoma, melanoma and Ewing's sarcoma were excluded from consideration, especially with reference to the frequency of occurrence of necrotic and fibrotic alterations. There was complete destruction of pattern in practically all of the leukemic nodes examined. This was usually associated with infiltration of immature myeloid, monocytic and lymphoid cells. The latter type of cells especially showed degeneration of the nuclei (chromatolysis, karyorrhexis and pyknosis). Grossly, most of the nodes were enlarged, moderately firm, grayish white on cut section and nonadherent to the surrounding structures. Moderate to severe collagenous thickening with occasional hyalinization was observed in the capsule in 20 cases. There were telangiectasia of capillaries, lymphatics and venules in 6 cases and associated focal fibrillary eosinophilic necrosis in 4. Atypical giant nuclei (fig. 5) were demonstrated in 20 cases, in 11 of which the nodes contained no areas of necrosis or fibrosis. The nuclear chromatin in these bizarre cells was usually increased in density, and prominent nucleoli with abnormal mitotic figures were also seen. The cytoplasm varied from light basophilic to dark eosinophilic and was nongranular; frequently the nucleus occupied the entire cellular structure. These cells resembled the atypical megakaryocytes produced as a result of irradiation. However, their increased diameter, the absence of light eosinophilic cytoplasm and their presence in other tissues of the body not associated with extramedullary hemopoiesis make this presumption somewhat doubtful. It seems more likely that they represent variants of reticulum cells, fibroblasts or immature myeloid elements produced as a result of multipolar mitoses. However, the possibility that they are produced by fusion of the nuclear remnants of related cells cannot wholly be excluded. Cervical, axillary, tracheobronchial, mesenteric, celiac and periaortic nodes and the lymphoid tissue of the ileum and the colon were studied. A comparison of the described atypical giant cell with the giant cell of untreated Hodgkin's disease can be made in figure 6.

Spleen.—Unlike that which is frequently encountered following severe roentgen irradiation, none of the spleens studied were smaller than normal. Thirty weighed over 300 Gm., the weight of the largest being 1,960 Gm. The external capsular surface was usually thickened and grayish white; in 4 instances it was adherent to the diaphragmatic surface—a phenomenon frequently noted in untreated leukemia. In most cases, on cut section the consistency was firmer and the

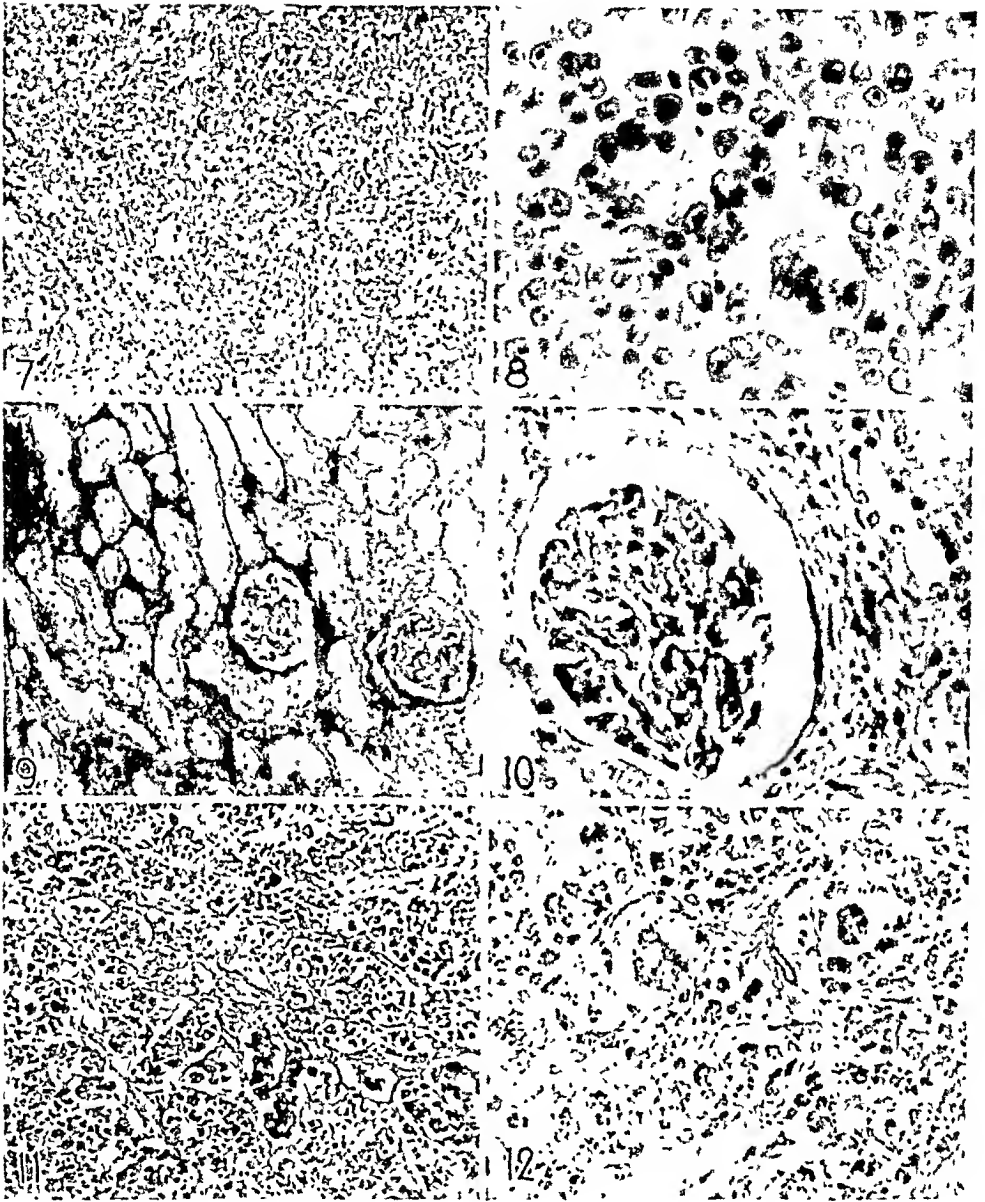


Fig. 7.—Spleen showing increased thickening of the basement membranes of sinusoids, with obliteration of some sinusoids and telangiectatic appearance of others. Notice the prominent, swollen endothelial cells lining these channels throughout and the focal area of eosinophilic fibrinous necrosis in the upper left hand corner. Hematoxylin and eosin; $\times 79$.

Fig. 8.—Spleen. Note the large multinucleated giant cell and the abnormal mitotic figures therein and in surrounding cells. The nuclei are eccentrically placed, and the cytoplasm is dark eosinophilic. Hematoxylin and eosin; $\times 340$.

Fig. 9.—Kidney of a 28 year old white man. Note the hyalinization and thickening of Bowman's capsule with slight thickening of the basement membrane of the glomerular tuft capillaries. Adjacent tubular structures show slight degenerative changes. Heidenhain-azocarmine stain; $\times 79$.

Fig. 10.—Kidney of a 26 year old white man. Note thickening and hyaline change in Bowman's capsule with similar alterations in glomerular capillaries. Hematoxylin and eosin; $\times 380$.

Fig. 11.—Pituitary gland showing moderate interacinous fibrosis with atrophy and necrosis of adjacent acidophilic and chromophobic cells. Hematoxylin and eosin stain; $\times 79$.

Fig. 12.—Pancreas of a 28 year old white woman. Marked interacinous collagenous thickening with fibrous tissue formation and hyalinization are illustrated. The pancreatic cells show varying degrees of degenerative changes.

color grayer than normal, and prominent trabeculae traversed the splenic pulp. Microscopically, there was masking of the normal pattern by the malignant cellular invasion. Occasional extramedullary hemopoietic foci were also seen. This type of hemopoiesis was especially prominent in those cases in which the marrow had been severely damaged. Only a few degenerating lymphocytes were visible around the central arterioles of the malpighian corpuscles. However, those alterations which are more closely related to radiophosphorus therapy are an increase in fibrous tissue involving the trabeculae and the sinusoidal and arteriolar walls, focal fibrinous necrosis and hyalinization of these structures, and an increase in the number of multinucleated giant cells (fig. 8). The connective tissue increase was demonstrated in 28 cases, and in 16 of these, zones of acidophilic necrosis were also shown. The thickening of the basement membrane of the sinusoids had obliterated some of these vascular spaces. Other sinusoids were telangiectatic (fig. 7). The fibrotic and hyaline changes involving the media and the adventitia of the central arterioles and occasionally the larger vessels had produced partial narrowing and almost complete obliteration of the lumens. There were also moderate to striking hyperplasia and swelling of the endothelial cells with formation of vesicular, foamlike cells⁷ in the intimal and medial coats. Similar changes were also observed in some of the blood vessels of the uterus (fig. 4), the marrow and the kidneys of patients in this series who were under 40 years of age. Occasionally there were whorls of fibrous tissue encircling portions of malpighian corpuscles. There was also a conspicuous deposit of hemosiderin in the red pulp.

Kidneys, Ureters and Bladder.—The renal changes observed after repeated intravenous injections of radioactive material can probably be attributed to the concentration that occurs in the nephron during the process of filtration and reabsorption. In addition, it has been previously determined⁸ that renal tissue is in itself moderately responsive to radiation. In 32 of the cases included in this series there were vascular and tubular renal alterations. Since it is difficult to distinguish changes secondary to primary vascular disease and changes due to beta radiation effect, only those 15 cases in which the patient was below 40 years of age were considered sufficiently characteristic to be placed in the latter category. The most characteristic histologic changes were thickening and fibrosis of the renal capsule and hyalinization and thickening of Bowman's capsule (figs. 9 and 10) with only rare involvement of the basement membrane of the glomerular tuft. Also observed were hyperemia, swelling, vacuolation and desquamation of the epithelium of the tubules, especially in the convoluted tubules. The blood vessels were not severely damaged but occasionally showed some thickening of the intima. Infrequently, the interstitial tissue was condensed and hyalinized. In a few of the ureters examined, there was histologic evidence of fibrosis. The various stages of the reaction of the bladder to radioactive phosphorus were characterized by varying degrees of hyperemia, occasional edema, desquamation of epithelial cells and increase in hyalinized connective tissue involving the mucosa, submucosa and muscularis. There was no history of catheterization or cystoscopic procedures in the cases in which the aforementioned microscopic changes were observed. No specific effects were seen in the prostate and seminal vesicles.

Endocrine System.—Pituitary Gland: Histologic study of this endocrine organ revealed a slight to moderate increase of connective tissue distributed between the acini (fig. 11). In 1 case there was such marked fibrosis that compression

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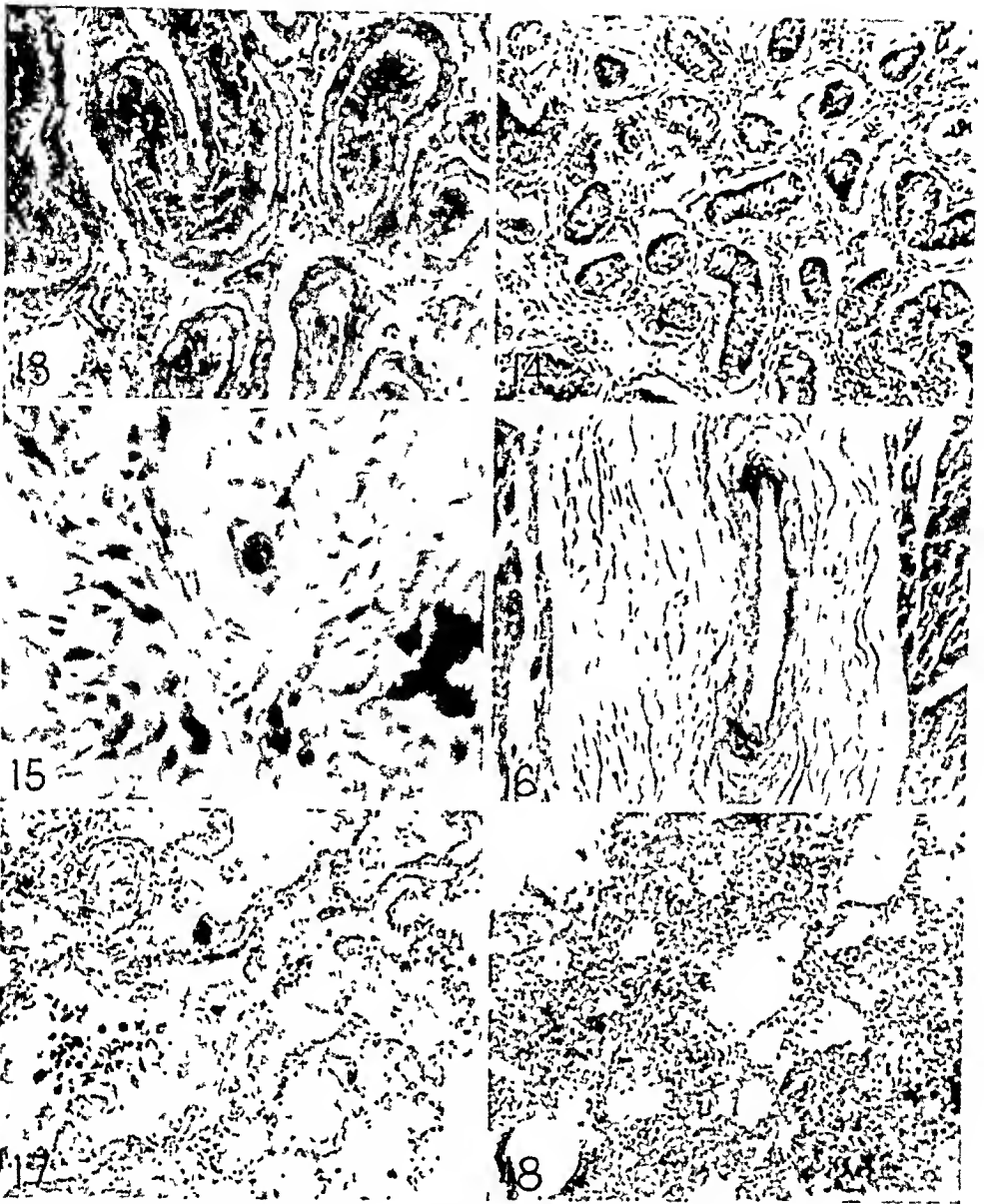


Fig. 13.—Testis of a 38 year old Negro man (no history of mumps or of testicular damage). Note the glassy hyalinization and thickening of the tunica propria and the basement membrane of each seminiferous tubule. No true spermatogenesis is visible. There is moderate edema of the interstitial tissues, with narrowing of the smaller vessels and almost complete disappearance of Leydig cells. Hematoxylin and eosin stain; $\times 79$.

Fig. 14.—Testis of a 13 year old white boy (no previous history of mumps or of testicular damage). There is interstitial fibrosis with thickening of the tunica propria and the basement membrane of tubules. The latter have retracted from the basement membranes, and there is practically no spermatogenesis seen. Arterioles in the interstitial tissue show hyaline changes in the media. Hematoxylin and eosin stain; $\times 79$.

Fig. 15.—Ovary of an 18 year old white woman (no history of mumps or of ovarian damage). The ovum shows degenerative changes involving the nucleus and the follicular epithelium. Hematoxylin and eosin stain; $\times 340$.

Fig. 16.—Heart of a 28 year old white man (no history of pathologic changes of coronary arteries or rheumatic heart disease). Note fraying and separation of medial and adventitial fibers, with zones of hyalinization, in a branch of the coronary artery. Hematoxylin and eosin stain; $\times 79$.

Fig. 17.—Lung showing degenerative metaplastic changes in the alveolar epithelium and thickening of the alveolar wall, with hyaline membrane formation and dilated alveoli. Note the hyaline changes in the medial coat of a small artery. Hematoxylin and eosin stain; $\times 79$.

Fig. 18.—Hyaline alterations and slight fibrosis in pulmonary parenchyma, with narrowing and obliteration of some alveolar sacs. A small hyaline thrombus partially occupies the lumen of a vessel. Hematoxylin and eosin stain; $\times 79$.

atrophy and necrosis of the adjacent eosinophilic and chromophobe cells were present. Other eosinophilic cells appeared swollen, the cytoplasm having an amorphous hyaline appearance. No associated clinical effects were observed.

Adrenal Glands: In 18 instances the capsules of both adrenal glands were markedly thickened by hyalinized connective tissue. The periadrenal arterioles showed similar hyaline degenerative changes in the medial coats. Because of the frequency of postmortem cellular changes occurring in these glands, it is difficult to distinguish minimal changes in the cortex. However, the greatest and most constant alteration was marked dissolution of cells and loss of cellular detail. These changes were observed in adrenal glands removed two to four hours after death.

Thyroid and Parathyroid Glands: No significant characteristic changes were observed in the small number of glands examined.

Pancreas: The same criterion of age was used in an effort to distinguish the fibrotic changes of age and associated vascular disease from alterations caused by radiophosphorus. Twenty-one pancreases (11 from patients below 40 years of age) showed moderate to marked interacinic and interlobular fibrosis with occasional hyaline changes in the collagen fibers (fig. 12). Three of these pancreases also revealed slight fibrosis of the islets. There were no duodenal ulcers and only minimal periductal reaction. There were edema of the collagen about the acini, vesiculation and desquamation of epithelial cells and occasional vacuolation and increased secretion of mucus by the ductal epithelium.

Reproductive Organs.—Testis: Of 16 testes from patients under 40 years of age, 11 showed varying degrees of destruction of the germinal epithelium. There were some tubules that showed almost complete disappearance of spermatocytes. Those that remained exhibited different stages of nuclear degeneration. In those testes showing the more severe changes, the only evidence of spermatogenesis that was observed consisted of fragmentary pyknotic nuclei. In practically all instances the seminiferous tubules showed thickening and varying degrees of hyalinization of the tunica propria and the basement membrane (fig. 13). Occasionally, amorphous groups of fused cells which resembled the Sertoli type were observed. There were also moderate to abundant edema of the interstitial tissue with diminution in the number of identifiable Leydig cells. In some cases the only significant abnormality was a mild degenerative change—for example, vacuolation of the cytoplasm of the tubular cells. A characteristic interstitial fibrosis, with practically no spermatogenesis in the tubules, was observed in still other testes (fig. 14).

Ovary: The most conspicuous alteration in ovarian tissue was the disappearance of the primary and graafian follicles. Those ova which remained either showed varying degrees of degeneration (fig. 15) or were replaced by a hyaline remnant. Those corpora lutea which were seen exhibited few significant changes. The interstitial tissue showed occasional areas of edema, lymphocytic infiltration or increased hyperemia, with telangiectatic vessels therein. These changes were observed in 6 of 7 ovaries studied, from patients who were under 40 years of age.

Heart.—Minimal changes were demonstrated in the myocardium and the coronary blood vessels of 14 of 21 patients under 40 years of age. These changes consisted of infrequent foci of granular and vacuolar degeneration of the myoplasm and the nuclei of individual cells. There were also occasional subepicardial and endocardial zones of hyalinized connective tissue. The vascular supply of the heart, especially the larger branches, showed moderate to severe fraying and separation of the medial and adventitial fibers, with small zones of hyalinization therein (fig. 16).

Lungs.—The pneumonitis caused by the beta radiation of P^{32} has been previously described as seen in experimental animals by Warren and Gates.¹⁰ The changes in the pulmonary tissue of 27 patients of the present series who were under 50 years of age were similar to the experimental findings and characteristic in 20 cases. The alterations ranged from moderate congestion, edema, lymphangiectasis, slight inflammatory cell infiltration and minimal degenerative metaplastic changes in bronchial and alveolar epithelium to well defined hyaline membrane formation (fig. 17), extreme thickening of alveolar walls, focal atelectasis, thickening of pulmonary vessels with associated swelling of collagen and hyaline degenerative changes therein. Moderate to severe fibrosis of pulmonary parenchyma was also observed (fig. 18). It is difficult to ascertain the relation of these changes to intercurrent infection, but the increase of connective tissue was not infrequently present in nonpneumonic, nonatelectatic and nonbronchiectatic pulmonary tissue. Occasionally various degrees of focal emphysema were associated with the hyaline membrane formation and the thickening of the wall.

Bone.—The significant features of the changes in bone can be summarized as follows: disappearance of osteoblasts and absence of osteocytes and lacunas. Occasionally the canaliculi were enlarged and irregular but reduced in number. Abnormal spaces sometimes appeared about the lamellas. Those osteocytes which remained showed pyknosis and vacuolation. In addition, it was difficult to distinguish any specific zone of demarcation between the normal and the irradiated bone. In reality, the reaction of adult bone to radioactive phosphorus was minimal when compared with the necrotic and more severe degenerative changes seen after heavy exposure to radium and roentgen radiation.

Skeletal Muscle.—Loss of cross striations, fragmentation of fibers, homogeneity of myoplasm and nuclear karyorrhexis were the characteristic microscopic changes seen in skeletal muscle fibers. Frequently Zenker's type of degeneration appeared. Occasionally, the less damaged fibers showed striking waviness of the myofibrils and loss of cross striations, which together accentuated the longitudinal fibrillation.

COMMENT

The increasing interest in temporarily radioactive isotopes, which are being used both as therapeutic agents and as tracers, necessitates a more detailed study of the microscopic changes noted in the body tissues as a whole. The fact that radioactive phosphorus is injected intravenously in most instances and that its 14.3 day half-life permits the tissues to be steadily irradiated for several weeks indicates that more diffuse cellular changes that are usually observed after local application of radium and roentgen radiation may be brought about. As can be determined by a study of the accompanying table, those tissues which utilize phosphorus rapidly and which also have a high phosphorus content, i. e., bone marrow, liver, spleen and lymph nodes, specifically take up higher concentrations of radioactive phosphorus than do normal tissues. Radioactive phosphorus is metabolized into nucleoproteins in the same manner as normal phosphorus (P^{31}). However, the rapidly proliferating cells take up much more radioactive phosphorus (P^{32}) than do normal, slow-growing cells. Therefore, proportionately higher concentrations of radioactivity are formed in those tissues preferentially involved in malignant lymphomas and primary polycythemia. Further

study of the table demonstrates an already obvious conclusion, i. e., that in almost every therapeutic application of radiation, normal tissues are affected as well as the intentionally irradiated focus of disease.² The profound sclerosing effect of radiophosphorus on the bone marrow has therefore led to variable hematologic complications in different patients, the peripheral and central response being independent of the dosage employed. According to Reinhard and his associates,³ when two or more of the cellular elements of the marrow were depressed in the same patient, the formed elements of the peripheral blood showed changes in the following order: The leukocyte level decreased first, the thrombocyte level second and the erythrocyte level last.

The most prominent alterations in immature marrow cells occurred in the megakaryocytes. These cells were either degenerated or completely absent in most of the marrow sections studied. Thus, from the clinical and the pathologic observations, the graphic representation of decreasing cellular sensitivity to radioactive phosphorus should be modified so that it would differ slightly from that previously proposed by Dunlap and Warren^{2b}—for example:

$$\left. \begin{array}{c} \text{Leukocytes} \\ \text{and} \\ \text{Leukopoietic} \\ \text{Tissue} \end{array} \right\} > \left. \begin{array}{c} \text{Megakaryocytes} \\ \text{and} \\ \text{Megakaryocytic} \\ \text{Tissue} \end{array} \right\} > \begin{array}{c} \text{Erythrocytes} \\ \text{and} \\ \text{Erythropoietic} \\ \text{Tissue} \end{array}$$

The urinary excretion of radioactive phosphorus, after intravenous injection, varies from 5 to 25 per cent during the first four to six days in patients with leukemia and polycythemia vera.⁹ However, approximately 25 to 50 per cent of the isotope is excreted by normal subjects within the same period. The difference in output is explained by Erf and Lawrence as being due to the quick fixation of radiophosphorus in the pathologic tissue and cells. This continued exposure of the renal parenchyma, with its extreme vascularity, to beta rays is probably the factor responsible for the thickening of Bowman's capsule and the associated tubular changes. Doub, Hartman and Bollinger¹⁰ regarded the kidney as the organ most susceptible to roentgen irradiation as far as anatomic changes and loss of function were concerned. They pointed out that direct irradiation of the exposed kidney could result both clinically and experimentally in nephritis with hypertension and therefore advised avoiding irradiation of the renal areas in young persons. This same question may be raised in the use of radioactive phosphorus during the relatively long life expectancy of the younger patients with primary polycythemia. Although it is true that long clinical and hematologic remissions may be produced in primary polycythemia by one course of therapy, repeated treatment may, perhaps, lead to severe irreversible renal vascular disease and subsequent hyper-

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tension. In fact, 11 of the 30 patients treated by Reinhard³ for polycythemia vera had a systolic blood pressure of 150 mm. or more of mercury, and 8 of these 11 had a diastolic pressure of 100 mm. or more of mercury. Furthermore, of the abnormal physical findings, hypertension responded least favorably to radiophosphorus therapy. Unfortunately, the patients with malignant lymphomas do not live long enough for a proper evaluation of renal and blood pressure changes. As yet I have had no opportunity to study the kidneys of patients with polycythemia treated for prolonged periods with radiophosphorus.

It is pertinent to mention the theory of anoxemia of the marrow as a cause of polycythemia vera.¹¹ Certainly, the oftener observed arteriolar narrowing and marrow fibrosis after radioactive phosphorus therapy would not serve to increase the oxygen intake of the medullary cavity. A similar question might also be raised as to the relative importance of the pulmonary alveolar and arteriolar thickening. Since these changes are frequently associated with the secondary type of polycythemia, it is possible that they make a relative contribution to the continuation of the anoxemia, to the persistence of the polycythemia and perhaps eventually to the development of right ventricular hypertrophy. However, an important consideration to be weighed against the foregoing objections, is the almost complete prolonged hematologic and symptomatic remission seen following this new type of radiation treatment.

Finally, serious consideration must be given to the changes in the testes and the ovaries of patients who are in the reproductive period of life. Observation of these organs confirms the possibility that spermatogenesis and oogenesis may decrease or disappear, with development of sterility, in young persons given radiophosphorus.

SUMMARY

The beta emanations of radioactive phosphorus (P^{32}) produce characteristic changes in many body tissues, alterations which vary in intensity but are similar to those produced by other types of radiation.

The lesions observed are the resultant of several diverse cellular, intercellular and vascular changes. Prominent specific features are varying degrees of cellular death, abnormal mitoses producing giant irregular nuclei, fibrosis, hyalinization of collagen and a vascular alteration characterized by thickening and hyalinization of small blood vessels.

There is selective localization of larger quantities of radioactive phosphorus and greater injury in the tissues most often involved by the neoplastic cells of malignant lymphomas and myelomas, i. e., marrow, liver, spleen and lymph nodes. Changes were also demonstrated in lungs, kidneys, gastrointestinal tract, ovaries and testes.

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BRAIN REPAIR

II. Role of the Lipids of the Brain in the Genesis of Gliosis

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THE CELLULAR processes which lead to repair after injury of the brain have been studied by a number of workers. The Macklins,¹ Russell² and Carmichael³ studied the function of the brain phagocytes, variously designated macrophages, gutter cells and microglia, and found them to be the major cellular participants in the acute reaction following injury by puncture. These phagocytes, which have been shown by Dunning and Furth⁴ to be members of the histiocyte-monocyte or so-called reticuloendothelial system, ingest cellular debris and erythrocytes in foci of necrosis or in the wounds caused by mechanical punctures of the brain. This process is most active during the first week, but the cells persist in wound tracks or other foci of injury for as long as several months to almost a year in some instances. That these same cells in the spleen exhibit strong proteolytic enzymatic activity has been demonstrated by Hicks and Opie,⁵ but in the brain the observation that the phagocytes persist at the site of injury, laden with lipids, for weeks and months, suggests that they are slow in digesting myelin and other fatty substances derived from brain tissue. During the acute reactive phase of the first week, there is also a proliferation of fibroblasts, the degree of which is determined by the amount of damage to the fibrous tissue accompanying blood vessels or the fibrous tissue of the meninges. Penfield and others⁶ showed that the amount of cicatrix forming in a puncture wound of the brain was proportional to the amount of damaged tissue left behind by the needle. A solid needle introduced meningeal

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connective tissue into the wound and the result was that in the wound track a fibrous core developed which often led to cicatricial contracture of the surrounding brain and meninges later on. A hollow needle, however, by removing a core of damaged tissue, left almost no injured tissue behind and produced little fibrous tissue damage and reaction. Such an injury healed with minimal scarring and contracture, a matter of importance in avoiding possible postoperative epilepsy. Gliosis, which is a proliferation of fibrillary astrocytes and a laying down of their fibrils, appears to be a secondary reaction in brain repair.⁷ Beginning changes in the astrocytes which lead to later proliferation have been observed around some experimental puncture wounds a few days after injury, but gliosis when it develops does not become conspicuous until about the third week, and the laying down of fibrils progresses for many weeks. It acts as a secondary repair mechanism in the brain. Around simple puncture wounds it may be almost absent or may develop at the margin of the track if that tissue has been injured. Carmichael and associates⁸ investigated the histologic sequence in the development of abscesses of the brain, devoting considerable study to the development of gliosis. They found that the primary response was that of the phagocytes and the proliferation of fibrous tissue around the injury. Astrocytes reacted secondarily around the primary process, producing a radially arranged mat of glial fibrils just outside the fibrous tissue wall and forming a less concentrated zone of loosely arranged astrocytes beyond the mat. This same pattern of reaction may occur around large hemorrhages and some other lesions. The stimulus to gliosis appears to be, therefore, a sublethal injury of the astrocytes, for their proliferation always occurs in a zone around a primary region of destruction or in a region where damage falls short of diffuse necrosis; as in anoxic, toxic, hypoglycemic and demyelinating processes.⁹ The sublethal injury may possibly be due to bacterial toxins diffusing outward from the primary site of an abscess, or to the relative anoxia which so often is associated with hemorrhage. A further example is seen in the astrocytes which in some cases of hyperinsulinism¹⁰ proliferate in regions of the fore-brain, which suffer from hypoglycemia sufficiently that many of the nerve cells¹¹ are destroyed but not so much that there is wholesale necrosis of brain tissue.

The sequence of events in repair of the brain after several general types of injury is summarized in the table.

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Brain Repair

	Simple Puncture Wound	Hemorrhage with Anoxia	Abcess	Injury Without Diffuse Necrosis, as in Anoxic, Hypoglycemic, Toxic and Demyelinating Processes
1st and 2d wk.....	Brain phagocytes ingest blood cells and elements of destroyed brain tissue. Fibroblasts and few capillaries grow from injured meningeal vascular sources into wound track. Phagocytosis reaches maximum during first week.	Brain phagocytes ingest blood cells and elements of destroyed brain tissue. Fibroblasts and few capillaries from injured vessels proliferate around hemorrhage, gradually walling it off	Reaction same as in hemorrhage, but more intensive, and polymorphonuclear cells from blood also accumulate in abscess in response to certain organisms, c. g., staphylococci	Brain phagocytes ingest destroyed myelin, dead neurons and processes. Proliferation of fibrous and vascular tissue is absent unless these elements are injured; then their reaction is usually slight
3d and 4th wk.....	Phagocytes persist; they contain lipids and blood pigments. Fibrosis attains maximum about 3d week. Gliosis (proliferation of astrocytes and their fibers) at margin of wound track is absent unless the surrounding tissue is injured	Phagocytes continue activity if lesion is large. Fibrous wall increases about hemorrhage. Proliferating astrocytes have become conspicuous around the fibrous wall, with radially arranged fibrils	Reaction as in hemorrhage, but injury to and proliferation of fibrous tissue are usually considerably greater, with formation of a thick wall. Gliosis has become conspicuous	Phagocytosis subsides unless there is continuing injury as in some demyelinating diseases. Proliferation of astrocytes (gliosis) is progressing
After 4th wk.....	Phagocytes containing lipids and blood pigment persist in wound track for months but diminish progressively. Fibrous tissue diminishes. Gliosis is not conspicuous unless surrounding tissue is injured. Fibrosis is the predominant feature of the end result	Phagocytes persist for weeks or months, depending on presence of persisting hemorrhage. Fibrous and glial wall about lesion with or without cyst formation is end result	Phagocytes persist for weeks or months, depending on balance between healing and necrosis. Fibrous and glial walls become prominent around abscess. End result is healing with fibrosis and gliosis, with or without cyst formation	End result is replacement of dead or degenerated tissue with fibrillary astrocytes: gliosis. Fibrosis is usually inconspicuous

As seen from the table, the gliosis developing in response to injury of the brain is a secondary reaction that is specific to the nervous system. The initial inflammatory reaction, characterized by a cellular exudate of phagocytes and followed by fibrosis and by granulation tissue in which fibroblasts predominate, is a nonspecific reaction seen in all tissues of the body.

The question arises whether gliosis is also stimulated directly by the injurious agent, or whether the stimulus arises secondarily from the liberation of lipid substances of destroyed nerve cells and their myelin sheaths. This problem is particularly interesting in regard to the demyelinating diseases of the nervous system. Bender¹² sought to throw some light on the problem by introducing several extracts of brain into the brains of dogs and studying the effect on repair after four weeks. Her results suggested that gliosis was stimulated to some extent by these substances, but the exact nature of the extracts used was not specified.

The following study was therefore undertaken to determine whether certain lipids of the brain when introduced by puncture into the brains of white mice would stimulate gliosis over and beyond that which is sometimes observed in simple puncture wounds. The lipids employed were sphingomyelin, lecithin, cholesterol and whole brain lipids. The wounds were compared histologically with simple puncture wounds, as controls, with the reaction to a foreign body (bone) and with the reaction to bacterial infection (avian tubercle bacilli).

CYTOLOGIC EXPERIMENTS

Young adult male and female white mice between the ages of 6 weeks and 3 months were used in all experiments. They were obtained from the stocks used for routine laboratory purposes at the Naval Medical School and the National Institute of Health. Under ether anesthesia and aseptic conditions, punctures of the brain were made with hypodermic needles to a measured depth of 4 mm. To insure uniform depth of the punctures a guard was used on each needle, set at 4 mm. The needle was introduced through the shaved skin and the skull into the middle of each cerebral hemisphere, puncturing the meninges, the cerebrum, the interbrain and often the lateral ventricle. Ventricular hemorrhage was never conspicuous. Suspensions of the lipids, avian tubercle bacilli and tiny bone fragments were introduced by the same technic. The suspensions of the lipids and the bone particles were made up in distilled water; those of the bacilli, in isotonic solution of sodium chloride. One one-hundredth of a cubic centimeter of suspension was injected when the needle had been introduced into the brain. The animals were killed rapidly with ether at varying intervals up to two hundred and seventy days after injury. The brain was removed and fixed at once in 10 per cent neutral solution of formaldehyde, U. S. P., or Zenker's solution with 5 per cent acetic acid and was stained with hematoxylin and eosin and Mallory's phosphotungstic acid-hematoxylin to emphasize astrocytic glial fibrils. An autopsy was done on each animal. No

12. Bender, L.: *Am. J. Path.* 1:657 and 667, 1925.

significant lesions were noted except those at the sites of brain puncture and lesions in the tuberculous animals, which will be described. The lungs, the livers and the spleens of tuberculous animals were examined microscopically after treatment with acid-fast stains as well as with hematoxylin-eosin and phosphotungstic acid-hematoxylin.

Simple Puncture of the Brain.—Twenty-six animals were divided into three series, and each cerebral hemisphere was punctured as described in the foregoing paragraph, three types of hypodermic needles being used: a standard hollow no. 23 needle, a standard no. 20 needle and an occluded no. 20 needle. Microscopically, the lesions produced by the three types of needles were remarkably uniform, and no noteworthy difference could be distinguished between the series, because each left behind the same amount of debris, and the lumen present in a needle in the absence of an occluding stylet was too small for a core of tissue to be removed in the manner employed by neurosurgeons. In a series of 14 animals the brain was punctured with the standard hollow no. 23 needle. One was killed four hours after injury and the rest on the second, third, fourth, sixth, eighth, eleventh, twelfth, fourteenth, seventeenth, twentieth, thirty-first, forty-second and sixty-second days after injury, respectively. In a second series of 6 animals the brain was punctured with the standard hollow no. 20 needle, and these mice were killed two, five, twelve, fifty, sixty and eighty days after injury. In a third series of 7 animals the brain was punctured with the occluded no. 20 needle, and 1 was killed on the fifth, 4 on the ninth, sixteenth, twenty-first and thirty-first days and 2 animals on the two hundred and seventieth day after injury.

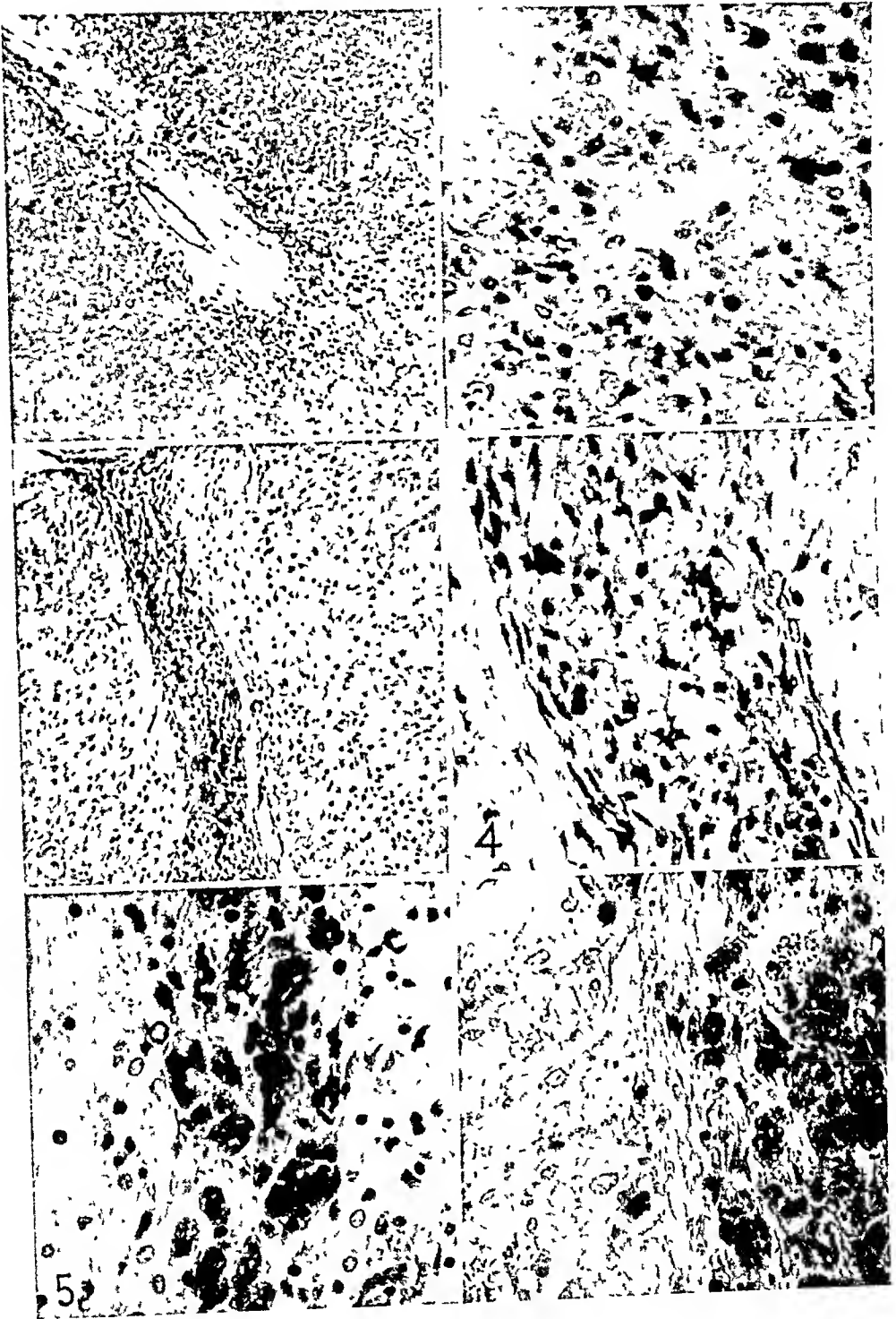
*Puncture with Introduction of Sphingomyelin.*¹³—One one-hundredth of a cubic centimeter of a freshly prepared sterile 2 per cent suspension of sphingomyelin was introduced by puncture with a no. 23 needle into each cerebral hemisphere of 4 mice. One was killed twelve days after injury, another on the twenty-second day and two on the two hundred and seventieth day after injury.

*Puncture with Introduction of Lecithin.*¹³—One one-hundredth of a cubic centimeter of a freshly prepared sterile 5 per cent suspension of lecithin was introduced by puncture with a no. 23 needle into each cerebral hemisphere of 4 mice. They were killed three, twelve, twenty-four and forty-four days after injury.

Puncture with Introduction of Cholesterol.—One one-hundredth of a cubic centimeter of a sterile approximately 2 per cent suspension of pure crystalline cholesterol was introduced by puncture with a no. 20 needle into each cerebral hemisphere of 3 mice. They were killed on the third, sixteenth and forty-fourth day after injury.

Puncture with Introduction of Whole Brain Lipids.—One one-hundredth of a cubic centimeter of a sterile suspension of whole brain lipid was introduced by puncture with a no. 23 needle into each cerebral hemisphere of 5 mice, which were killed on the third, twelfth, twentieth, thirty-second and forty-first days after injury. The suspension was prepared from the aseptically removed brain of a normal white mouse, which weighed 0.4 Gm. This was macerated and extracted for thirty minutes in the cold with 10 cc. each of absolute ethyl alcohol and ether, and filtered. The filtrate was evaporated at 40 C. with an air jet, and the residuum

13. These phosphatides were prepared at the Naval Medical School as a by-product of Kahn test antigen. The terms "sphingomyelin" and "lecithin" represent specific closely related groups of substances rather than single chemical entities (Page, I. H.: *Chemistry of the Brain*, Springfield, Ill., Charles C Thomas, Publisher, 1938.)



FIGURES 1 TO 6
(See legends on opposite page)

suspended in 1 cc. of distilled water. On the assumption that fresh brain is roughly 10 per cent lipids, the suspension was estimated to be about 4 per cent lipid.

Puncture with Introduction of Foreign Body (Bone Particles).—One one-hundredth of a cubic centimeter of a sterile suspension of bone particles was introduced with a no. 23 needle into each cerebral hemisphere of 5 mice. They were killed three, twelve, twenty, thirty-two and forty days after injury. The suspension of bone particles was made by grinding aseptically a piece of the skull bone of a normal white mouse in a mortar and suspending it in sterile distilled water. The resulting suspension was milky, and the particles passed through the no. 23 needle.

Puncture with Introduction of Avian Tubercle Bacilli.—One one-hundredth of a cubic centimeter of a suspension of avian tubercle bacilli was introduced with a no. 23 needle into each cerebral hemisphere of 4 mice. They were killed twenty hours, five days, twelve days and forty-eight days after injury. The suspension was prepared under aseptic conditions and made up in isotonic solution of sodium chloride from a six week old culture of virulent avian tubercle bacilli, grown at the National Institute of Health (Phipp's avian strain), and contained 1 mg. of bacilli per cubic centimeter.

RESULTS

Simple Puncture.—The histologic sections of the series of wounds caused by simple puncture of the brain showed virtually the same cytologic sequence of events described by others. After the first forty-eight hours, when hemorrhage with some fibrin filled the track formed by the needle, an exudate of phagocytes accumulated at the margins of the track and ingested the red blood cells and cellular debris. Polymorphonuclear leukocytes were extremely rare. This phagocytic reaction attained a maximum by the end of the first week. (See figures 1 and 2.) The chief cellular participant was the mononuclear phagocyte, which most observers believe is derived from the microglia of the

EXPLANATION OF FIGURES 1 TO 6

Fig. 1.—Wound track six days after simple puncture of the brain; $\times 81$; phosphotungstic acid-hematoxylin. The track contains blood with some fibrin, and in its margin there are large numbers of phagocytes containing lipid and a few hemosiderin granules. The surrounding tissue is intact and contains some phagocytes en route to the wound track.

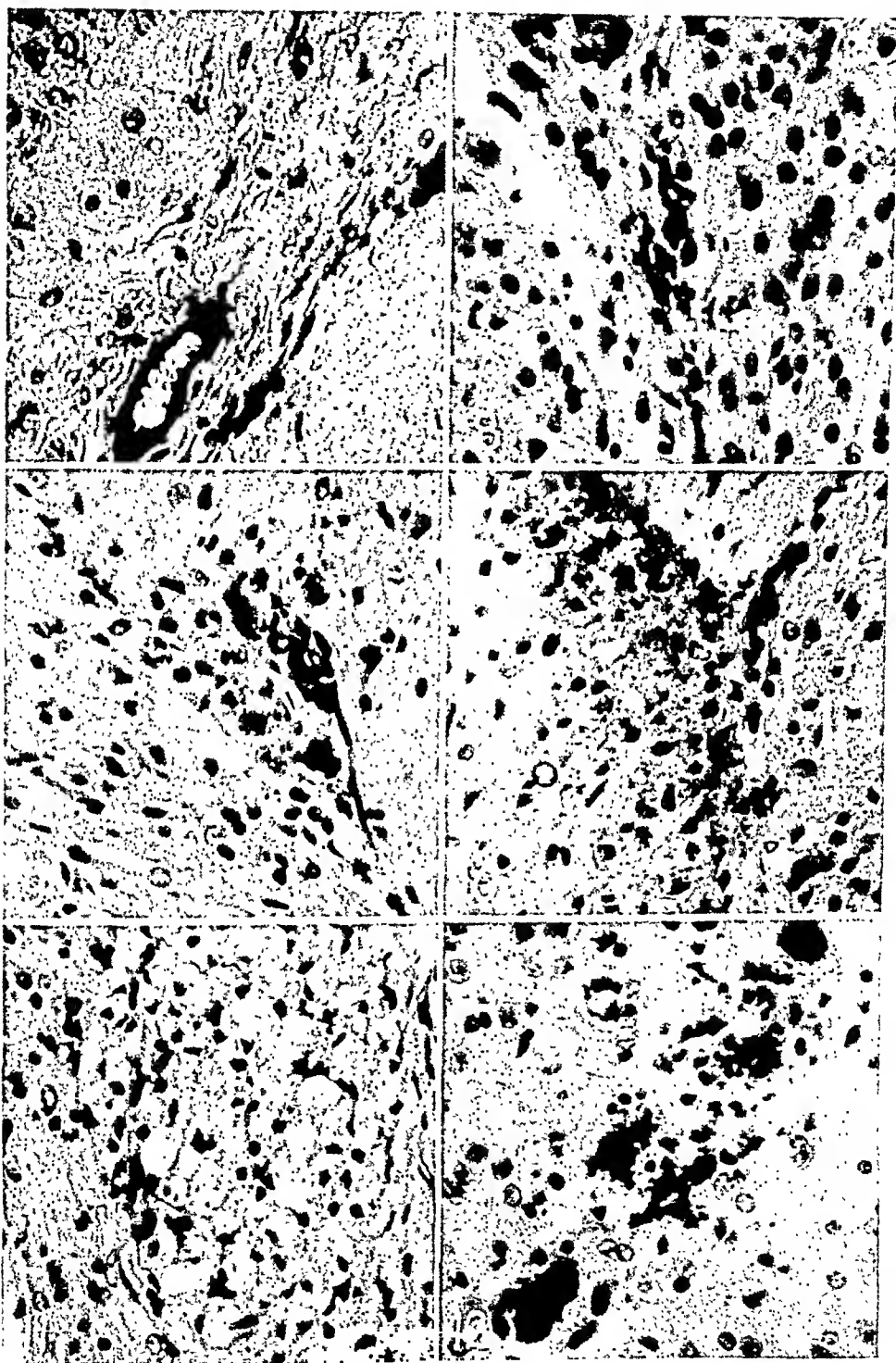
Fig. 2.—Lower end of the track seen in figure 1, $\times 324$, to show phagocytes.

Fig. 3.—Wound track twenty-one days after simple puncture; $\times 81$; hematoxylin and eosin. A loose core of fibrous tissue extending into the track from the meninges is seen. The track is packed with phagocytes containing lipids and hemosiderin. These two substances are irregularly distributed in the cells.

Fig. 4.—The midportion of the track seen in figure 3; $\times 324$. No astrocytic glial reaction is seen around the wound track.

Fig. 5.—Wound track thirty days after simple puncture; $\times 324$; hematoxylin and eosin. The track is composed of phagocytes and a loose core of connective tissue. Sections cut to show different parts of the track showed the usual irregular distribution of hemosiderin and lipids in the phagocytes.

Fig. 6.—Wound forty-two days after simple puncture, $\times 324$; phosphotungstic acid-hematoxylin. The appearance is virtually the same as that at thirty days. Hemosiderin is prominent in this portion of the track, but lipids constituted the major part of the phagocytosed material in other portions. No gliosis is evident.



FIGURES 7 TO 12
(See legends on opposite page)

brain and the monocytes of the blood stream. That these cells multiplied at the site of injury was evidenced by occasional mitotic figures in them, a fact recorded also by others. The possibility that they were formed in situ from the connective tissue about the blood vessels or from lymphocytes¹⁴ was not evident from these studies. By the end of the first week the cytoplasm of these phagocytes became heavily laden with lipids and blood pigment, some cells containing more of one substance than the other; with their included material some of them persisted in the wound tracks for as long as two hundred and seventy days. However, after the first few weeks there was progressive diminution of the number of such cells present.

Fibrous tissue proliferation developed *pari passu* with the acute phagocytic response. At first, the connective tissue fibers were found in association with the ingrowth of a few capillaries, which began within the first week. Neither this capillary ingrowth nor the pericapillary fibroblastic response was conspicuous. There was, however, a conspicuous ingrowth of fibrous tissue from the injured meninges overlying the lesion from the first few days after injury. By the second and third week it was sufficient to form a loose, irregular core of fibrous tissue, which occupied the wound track and was filled with persisting phagocytes. This picture continued through the thirtieth, forty-second, fiftieth, sixtieth and eightieth days, diminishing gradually. (See figures 3 to 7.)

14. Kulouch, F., Jr.: Am. J. Path. 15:413, 1939.

EXPLANATION OF FIGURES 7 TO 12

Fig. 7.—Wound two hundred and seventy days after simple puncture of the brain; $\times 324$, phosphotungstic acid-hematoxylin. It consists of a few fibrous tissue strands and scattered phagocytes containing hemosiderin and little lipid. No gliosis is present.

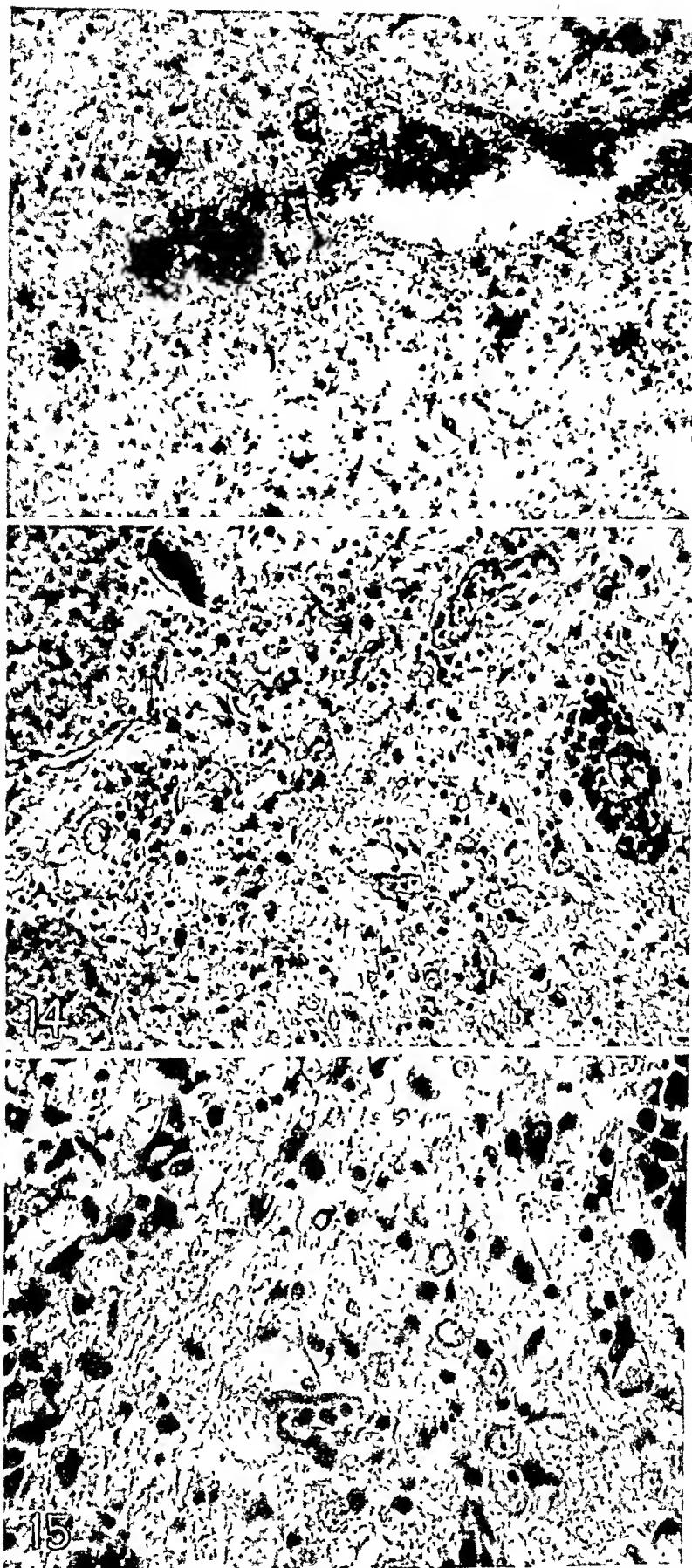
Fig. 8.—Wound two hundred and seventy days after puncture with introduction of sphingomyelin; $\times 324$; hematoxylin and eosin. The appearance is virtually the same as that of the wound shown in figure 7. No gliosis is present.

Fig. 9.—Wound forty-four days after puncture with introduction of cholesterol; $\times 324$; hematoxylin and eosin. It is shown in cross section and is composed of a few strands of fibrous tissue and phagocytes containing lipid and hemosiderin granules. No gliosis is evident.

Fig. 10.—Wound thirty-two days after puncture with introduction of whole brain lipids; $\times 324$; hematoxylin and eosin. The track consists of strands of loose fibrous tissue and phagocytes containing lipid and hemosiderin granules. No gliosis is evident.

Fig. 11.—Wound forty-four days after puncture with introduction of lecithin; $\times 324$; hematoxylin and eosin. The track is composed of phagocytes containing lipids and hemosiderin granules and a loose meshwork of connective tissue. There is no gliosis.

Fig. 12.—Wound thirty-four days after puncture with introduction of bone particles; $\times 324$; hematoxylin and eosin. This section shows the deep end of the track, where fibrosis and reaction to tissue injury are minimal. The bone particles are exciting almost no reaction. There is no gliosis.



FIGURES 13 TO 15
(See legends on opposite page)

By the two hundred and seventieth day following the injury produced by the puncture, the fibrous track had narrowed to a few strands of connective tissue intermingled with a few pigmented phagocytes. This blood pigment was rarely seen outside the phagocytes, which contained little lipid at this stage.

Throughout the entire course of the experiment, the uninjured brain tissue seen in sections around the wound tract showed little or no response to the injury. There was almost no proliferation of astrocytes. Glial fibrils could be seen only extremely rarely in some wound tracks after the third week, and were longitudinally disposed at the margins. They were so infrequent as hardly to justify the use of the term gliosis. The narrowing of the track was attributed to the gradual disappearance of phagocytes and the shrinking and disappearance of fibrous tissue. This was reflected by some contracture of the surrounding brain toward the track seen after the sixth week.

Puncture with Introduction of Sphingomyelin, Lecithin, Cholesterol or Whole Brain Lipids.—The introduction of the various lipids, sphingomyelin, lecithin, cholesterol and whole brain lipids, did not alter the response from that seen with simple puncture. (See figures 8, 9, 10 and 11.) Again, the sequence of events was destruction of brain tissue with hemorrhage, phagocytosis and fibrosis. There was no evidence of cholesterol crystals or of the other lipids in the wound tracks. In none of the sections was there evidence of astrocyte proliferation with formation of glial fibrils. The lipids introduced did not, therefore, stimulate gliosis in the tissue surrounding the wound tracks and seemed to be without effect on brain tissue.

Puncture with Introduction of Bone Particles.—The bone particles deposited in the wound track after puncture did not alter the sequence of events. They remained in place without exciting a reaction greater than that seen in the control puncture wounds; the phagocytic and

EXPLANATION OF FIGURES 13 TO 15

Fig. 13.—Wound forty-eight days after puncture with introduction of living avian tubercle bacilli; $\times 100$; phosphotungstic acid-hematoxylin. The wound track is filled with phagocytes containing lipid, blood pigment and many acid-fast bacilli. At the margins of the track there is some granulation tissue, toward which there are somewhat radially arranged astrocyte fibrils. In the zone around the wound track there are scattered plump fibrillary astrocytes. This zone shows little other change, the usual structure with some nerve cells being still evident.

Fig. 14.—A portion of the track shown in figure 13; $\times 200$. The phagocytes in the wound track are seen to the left, and loose granulation tissue appears to the right of them, merging into the surrounding brain, which shows considerable proliferation of astrocytes and their fibrils. A small blood vessel to the right, in this zone, shows perivascular lymphoid cells.

Fig. 15.—A portion of the section shown in figure 14, $\times 400$, to show astrocyte proliferation.

fibroblastic response progressed and began to subside during the nearly six weeks of observation. Figure 12 shows several particles at the edge of the lower end of one of the tracks. There is a minimum of cellular response to these particles. No gliosis was stimulated.

Puncture with Introduction of Avian Tubercle Bacilli.—Avian tubercle bacilli altered the response to injury considerably when introduced into the brain by puncture. (According to Rich,¹⁵ white mice are moderately susceptible to avian tuberculosis.) In the animal killed forty-eight days after puncture the wound tracks were filled with phagocytes containing lipids, blood pigment and acid-fast bacilli in large numbers. There were fibroblasts scattered in the track, and at the margins they were associated with a moderate number of new capillaries. Just at the outer edge of this zone there were fairly numerous astrocytes with proliferated fibrils arranged in somewhat radial fashion toward the wound track. A number of scattered plump fibrillary astrocytes were seen in a broad zone around the wound track where tissue destruction was minimal as evidenced by persistence of some nerve cells and most normal structures. (See figures 13, 14 and 15.) This gliosis was attributed to toxins diffusing into this zone, sufficient to cause sublethal injury but little necrosis. The animals killed twenty hours, five days and twelve days after the introduction of tubercle bacilli showed wound tracks comparable to those of the controls, but the phagocytes also contained acid-fast bacilli. The five day animal showed a few metastatic tuberculous foci in the liver and the spleen.

CONCLUSIONS

The experiments described, coupled with common observation, point to direct injury of astrocytes as the stimulus necessary for the production of gliosis. Astrocytes proliferate and lay down fibrils in regions of the brain that suffer damage from toxins and injurious processes sufficient to inflict minimal damage and kill a few nerve cells and their processes. The lipids liberated by destruction of brain tissue do not seem to stimulate gliosis. Were the lipids a stimulating factor, glial proliferation at the margin of the tracks ought to have been evident in both the lipid and the simple puncture experiments. Proliferation of astrocytes is a secondary healing mechanism. Astrocytes, unlike fibroblasts in association with capillaries, cannot organize dead tissue or hemorrhage. The fibroblasts may replace and fill up a defect caused by complete necrosis, as in an abscess, but the astrocytes cannot. Thus they are seen at the edges of areas of complete necrosis; only in places where necrosis is incomplete do they serve as replacement.

15. Rich, A. R.: Pathogenesis of Tuberculosis, Springfield, Ill., Charles C Thomas, Publisher, 1944, chap. 5, pp. 120 and 124.

SUMMARY

Studies were undertaken to determine whether (a) the setting free of lipids in foci of destruction of the brain or (b) direct injury of astrocytes is the stimulating factor in the production of gliosis.

The histologic sequence of events following simple punctures of the brains of white mice was compared with that seen after punctures in which sphingomyelin, lecithin, cholesterol and whole brain lipids were introduced into the wound tracks. A similar comparison was made with punctures into which bone particles and avian tubercle bacilli were introduced.

The histologic sequences following simple puncture of the brain, puncture with introduction of the brain lipids, and puncture with introduction of bone particles were the same: initial phagocytosis of damaged tissue and hemorrhage, followed by fibrosis. The lipids and the bone particles did not alter the response seen after simple puncture. Gliosis was almost entirely absent.

The sequence following puncture of the brain with introduction of avian tubercle bacilli was altered from that seen in the simple puncture and puncture with introduction of the brain lipids. Not only were phagocytosis and fibrosis evident but also, at the margins and in the zone around the wound tracks, proliferation of astrocytes and their fibrils.

It was concluded that proliferation of astrocytes and their fibrils is dependent on direct injury of the astrocytes and is not due to any stimulating action of the lipids liberated in the destruction of brain tissue.

EXPERIMENTAL STUDY OF PURPURIC MENINGOCOCCEMIA IN RELATION TO THE SHWARTZMAN PHENOMENON

With Discussion of Meningococcic Purpura, the Waterhouse-Friderichsen Syndrome
and Bilateral Renal Cortical Necrosis

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MENINGOCOCCIC bacteremia characterized by cutaneous purpura has attracted much attention by virtue of its frequently fulminating course, its striking cutaneous manifestation, its unpredictable selectivity and finally the occasional involvement of the adrenal glands by necrosis and hemorrhage.

Despite excellent descriptions¹ of these lesions, their genesis remains a matter of hypothesis. In 1904 Langmead² suggested that the lesions might be due to the blood-borne toxin of an unknown disease agent. MacLagen and Cooke³ proposed that the meningococcus selectively affects tissues of ectodermal origin. Impressed by the presence of eosinophilic leukocytes in various viscera, Glanzmann⁴ discussed the possibility of an anaphylactoid reaction to the toxins of a septic state. Sachs⁵ expressed the belief that the simultaneous presence of adrenal hypoplasia and the thymicolymphatic state exerted an important influence on the course of the disease. Schwartzman⁶ proposed that the disease might be a resultant of the Schwartzman phenomenon. György and co-workers⁷ suggested as a remote possibility that an acute bacterial septic state was superimposed on a nutritional deficiency. Jones,⁸ from his study of 5 nonfatal cases, concluded that an allergic constitution might

From the Department of Pathology, Duke University School of Medicine.

1. (a) Banks, H. L., and McCartney, J. E.: *Lancet* **1**:219, 1942; (b) **1**:771, 1943. (c) Herbut, P. A., and Manges, W. E.: *Arch. Path.* **36**:413, 1943.

2. Langmead, F.: *Lancet* **1**:1496, 1904.

3. MacLagen, P. W., and Cooke, W. E.: *Lancet* **2**:1054, 1916.

4. Glanzmann, E.: *Jahrb. f. Kinderh.* **138**:49, 1933.

5. Sachs, M. L.: *Ann. Int. Med.* **10**:1105, 1937.

6. Schwartzman, G.: *Phenomenon of Local Tissue Reactivity*, New York, Paul B. Hoeber, Inc., 1937.

7. György, P.; Goldblatt, H.; Miller, F. R., and Fulton, R. P.: *J. Exper. Med.* **66**:579, 1937.

8. Jones, B. B.: *Virginia M. Monthly* **72**:32, 1945.

be a predisposing factor in the genesis of the disease. None of the theories mentioned is supported by adequate experimental or clinical evidence.

In 1928 Shwartzman⁹ demonstrated a necropurpurogenic factor in meningococci. This was accomplished with bacterial culture filtrates inoculated intradermally (preparatory) and followed, optimally, twenty-four hours later with an intravenous (reacting) injection of the same filtrate. Within three to four hours after the reacting injection (also called the provocative inoculation) the skin at the site of preparation became hemorrhagic and then necrotic.

Because it was believed that a demonstrable relationship might exist between the lesions of meningococcic purpura and the Shwartzman phenomenon, the following three experiments were undertaken.

EXPERIMENT 1

Method.—Meningococci (strain P₁), isolated from the blood of a patient who had developed widespread cutaneous purpura, were grown on dextrose-starch-agar slants for twenty-four hours. The growth on each slant was washed off with 1 cc. of sterile isotonic solution of sodium chloride. The washings were centrifuged until the supernatant fluid was clear, whereupon it was decanted and sterilized by heating for fifteen minutes at 60 C. This material is hereafter referred to as "sterile supernatant α ." The remaining meningococcic suspension was made up to its original volume or more by the addition of isotonic solution of sodium chloride and centrifuged a second time until the supernatant fluid was clear. The second decanting was followed by a second dilution with isotonic solution of sodium chloride centrifugation and further decanting. The organisms were resuspended for the last time in isotonic solution of sodium chloride, the original volume being restored. The resulting suspension of organisms constitutes the "live washed meningococci" used in all three parts (A, B and C) of experiment 1. After removal of the required amount of material the remainder of the suspension was heated for fifteen minutes at 60 C., resulting in death of the bacteria. This constitutes the "dead washed meningococci." The inoculation of materials thus produced was always carried out within a few minutes after their preparation.

Each step of the procedures was controlled by culture of the inoculums just before use, insuring respectively the sterility of the material and the viability or the death of the organisms.

Supernatant β (table 1) was made up in one batch by a modification of Shwartzman's method. The meningococci were grown on dextrose-veal-agar in Kolle flasks for twenty-four hours, and the growth of each flask washed off with 3 cc. of isotonic solution of sodium chloride. The pooled washings were centrifuged until the supernatant was clear. It was then decanted, heated for fifteen minutes at 60 C. and stored in stoppered sterile bottles. Its potency was tested by producing the typical Shwartzman reaction in a number of rabbits. No attempt was made to titrate the strength of the material.

Each of the three parts of experiment 1, A, B and C, as illustrated in table 1, was carried out on 15 rabbits. The animals, not selected by sex or subspecies,

9. Shwartzman, G.: Proc. Soc. Exper. Biol. & Med. 26:207, 1929.

averaged between 5 and 7.5 pounds (2 and 3 Kg.) and were divided into three groups of 5: E, F and G.

Approximately twenty-four hours before the first inoculation, the abdominal hair of the rabbits was clipped and then depilated with barium sulfide.

The intradermal inoculation, in all instances 0.15 cc. of material, was followed approximately twenty-four hours later with an intravenous injection of 1 cc. of material. The results of the experiment are illustrated in table 1.

Results.—Three animals, one each of groups BG, CF and CG, were excluded because of imperfect intravenous injections. Characteristic cutaneous lesions are depicted in figure 1.

Reading the results horizontally (table 1), i. e., AE, AF and AG, permits comparison of the preparatory potency of the various substances.

TABLE 1 (Experiment).—Table Showing That the Local Shwartzman Reaction May Be Prepared and Provoked in Rabbits by Living, as Well as Dead, Meningococci or Their Supernatant Fluid

Rabbit Group E		Rabbit Group F		Rabbit Group G	
Live Washed Meningococci	Reaction	Sterile Supernatant Fluid	Reaction	Dead Washed Meningococci	Reaction
Part A {	Ld. Meningococci	Ld. Supernatant a	{	Ld. Meningococci	{
	+				
	+				
	+				
Part B {	Lv. Supernatant β	Lv. Supernatant β	{	Lv. Supernatant β	{
	+				
	+				
	+				
Part C {	Ld. Supernatant β	Ld. Supernatant β	{	Ld. Supernatant β	{
	+				
	+				
	+				
Part D {	Lv. Meningococci	Lv. Supernatant a	{	Lv. Meningococci	{
	+				
	+				
	+				
Part E {	Ld. Meningococci	Ld. Supernatant a	{	Ld. Meningococci	{
	+				
	+				
	+				
Part F {	Lv. Meningococci	Lv. Supernatant a	{	Lv. Meningococci	{
	+				
	+				
	+				

Ld. = Intradermal inoculation (preparatory).

Lv. = Intravenous inoculation (provocatory).

+

0 = no Shwartzman reaction.

⊕ = rabbit died too soon for reaction to manifest itself.

I = original experiment.

II = check experiment.

It is apparent that living and dead washed meningococci are capable of preparing the skin of rabbits, while the sterile supernatant is not. In a further experiment, not included in this paper, it was demonstrated that this inability is due not to the absence of the preparatory factor but to its low concentration in the fluid.

BE, BF and BG show that all three materials may provoke the phenomenon. Since Shwartzman⁶ has demonstrated that meningococcic filtrates are more potent as provocative than as preparatory agents, the results are in keeping with expectations.

E and G of experiment C were repeated and are labeled, respectively, CE II and CG II. The washed organisms, both living and dead, are demonstrably capable of preparing and eliciting the Shwartzman phe-

nomenon without the agency of filtrates. As in the case of AF, repetition of CF, with more concentrated preparations being utilized, resulted in successful production of the phenomenon.

Reactions produced with living organisms appeared, on the whole, somewhat larger and more intense than those elicited with dead meningococci. These results support those of Pabst and Branham,¹⁰ who found that heating potent filtrates, even at moderately low temperatures, appeared to decrease their potency.

While gross examination of the various lesions shows no appreciable differences other than those of size, section through the centers reveals

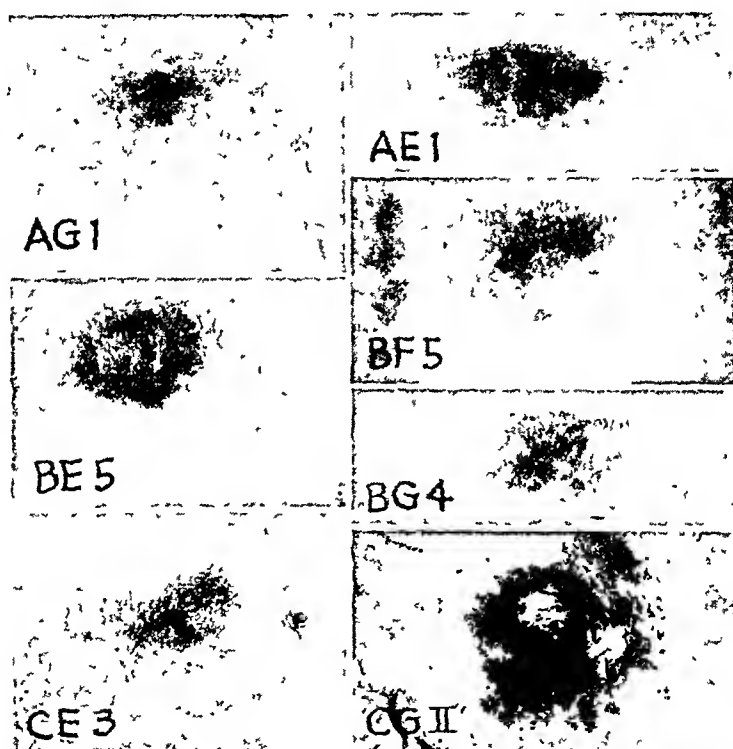


Fig. 1 (experiment 1).—Cutaneous Shwartzman reactions illustrating table 1. Each reaction is designated by reference to the part of the experiment (A, B or C) and to the rabbit group (E, F or G) and to the individual rabbit (1 to 5).

to the unaided eye that in many of the lesions of groups AE, AG, CE and CG an abscess has formed in the corium at its junction with the subcutaneous fat. Histologically (fig. 2) the cavity is crowded with polymorphonuclear leukocytes and some macrophages. The surrounding tissues are edematous and infiltrated by polymorphonuclear leukocytes. Adjacent blood vessels, both veins and arteries, are involved by the inflammation, presenting characteristic thrombophlebitis and thromboarteritis. Hemorrhage of varying degree is prominent. In almost

10. Pabst, A. M., and Branham, S. E.: Pub. Health Rep. 48:639, 1933.

all instances the covering epidermis is still present but shows edema and an occasional infiltrating leukocyte.

The lesions produced in subgroups BE, BF and BG are similar in all respects to those described except for the absence of any but micro-abscesses. Acute cellulitis and thrombophlebitis and thromboarteritis with hemorrhage are seen in all positive reactors. The lesions are identical with those described by Schwartzman,⁶ Apitz,¹¹ Moritz¹² and others and require no further description.

At necropsy 1 animal was selected from each group for more complete histologic study. Sections were removed from heart, lungs, liver,



Fig. 2.—Cellulitis, thrombophlebitis and abscess formation characterizing the local cutaneous Schwartzman reaction (experiment 1); $\times 52$.

spleen, kidneys and adrenal glands. The spleen showed the most consistent changes; the lymphoid follicles were enlarged and in many instances infiltrated by groups of polymorphonuclear leukocytes, among which were numerous pseudoeosinophilic leukocytes. Macrophages filled with cellular debris were likewise seen within the follicles (fig. 3 A). The sinusoids, particularly those about the follicles, contained numerous polymorphonuclear leukocytes and occasional thrombi. They

11. Apitz, K.: *Ztschr. f. d. ges. exper. Med.* **89**:699, 1933.

12. Moritz, A. R.: *J. Exper. Med.* **66**:603, 1937.

were lined by hyperplastic reticuloendothelial cells. Since these changes manifested themselves eccentrically about the follicles, the result was a form of inflammatory crescent. The changes encountered in the other organs, with one exception, were similar to those described in great detail by Apitz,¹³ Schwartzman, Klemperer and Gerber¹⁴ and Gerber,¹⁵ namely, occasional foci of acute myocarditis and necrosis (fig. 3 *B*) and focal necrosis of the liver (fig. 3 *C*). The lung of rabbit CE₄ presented the exception noted. Figure 3 *D* depicts a small pulmonary artery showing an unusual type of arteritis. A segment of the vessel

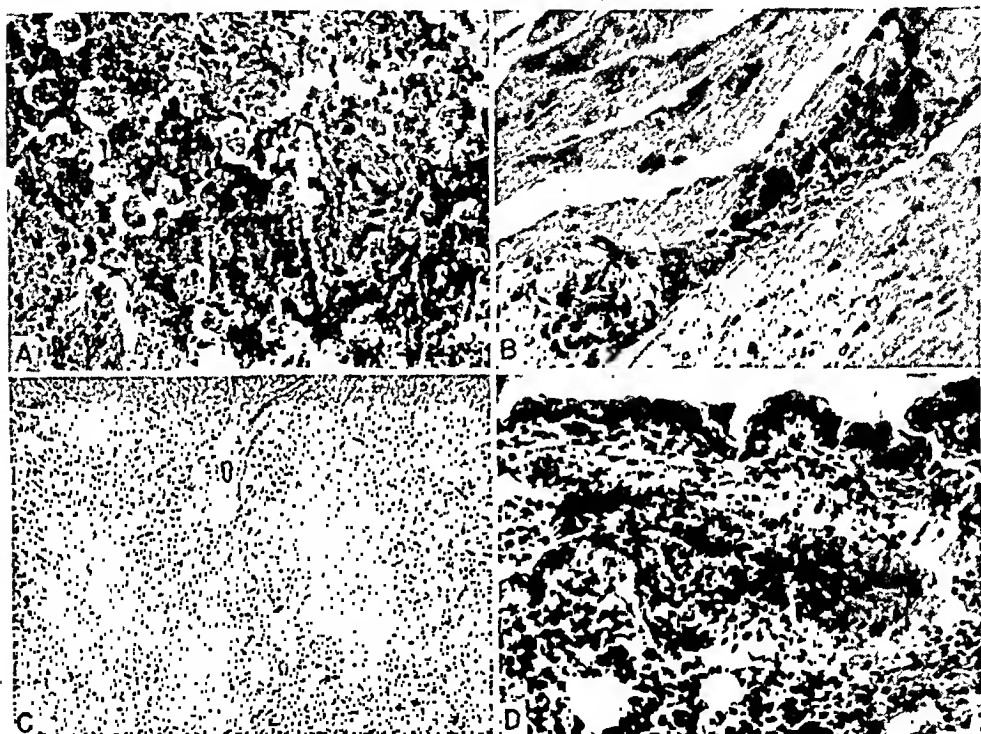


Fig. 3.—Visceral lesions accompanying production of the local cutaneous Schwartzman phenomenon (experiment 1); $\times 181$. *A*, hyperplastic splenic follicle revealing debris-laden macrophages and infiltrating polymorphonuclear leukocytes. *B*, necrosis of myocardial fibers. *C*, focal hepatic necrosis. *D*, pulmonary arteritis.

reveals necrotic hyaline muscularis infiltrated by monocytes, among which are polymorphonuclear leukocytes. The intima is thickened by the presence of the same cell types. These obstruct the lumen. The adventitia and the periadventitia are likewise the site of a similar exudate.

13. Apitz, K.: *Virchows Arch. f. path. Anat.* **293**:1, 1934.

14. Schwartzman, G.; Klemperer, P., and Gerber, I. E.: *J. A. M. A.* **107**: 1946, 1936.

15. Gerber, I. E.: *Arch. Path.* **21**:776, 1936.

EXPERIMENT 2

Method.—While the method of Shwartzman is, beyond doubt, effective in estimating the potency of a given bacterial filtrate, it appeared to be of dubious value as a means of comparing the potency of one strain of meningococci with another. With different strains grown under uniform conditions there resulted at times gross discrepancies in estimates of numerical population. Since the potency of a given filtrate is directly related to the number of organisms in the culture, it was decided to utilize quantitative methods for assaying the potency of 18 meningococcal strains collected from various sources. Dead bacteria were used throughout (experiment 1). To obtain approximately equal numbers of organisms the following experiment was carried out: Meningococci of a single strain were planted on three dextrose-starch-agar slants and incubated for twenty-four hours. The growth was then washed off with 1 cc. of sterile saline solution. The organisms in the pooled washings were promptly killed by heating at 60 C. for fifteen minutes and twice washed as in experiment 1. After the last supernatant fluid had been decanted, 2 cc. of isotonic solution of sodium chloride was added, and the organisms were resuspended and centrifuged

TABLE 2 (Experiment 2).—Production of Purpurogenic Substance by Eighteen Meningococcal Strains

Potency > P ₁	Series Group	Potency = P ₁	Series Group	Potency < P ₁	Series Group
BG ₁ •.....	II	P ₁ •.....	II	D ₁ •.....	II
BG ₂ •.....	I	F ₁ •.....	I	W ₁ •.....	II
G ₁ •.....	IIa	D ₁ •.....	II	W ₂ •.....	I
D ₁ •.....	I	56•.....	II	51•.....	II
P ₂ •.....	I	G ₂ •.....	IIa	55•.....	I
44B.....	III	70•.....	I		
		G ₂ •.....	I		

• = organisms were recovered from cases of meningococcal purpura.

○ = organisms were recovered from cases of nonpurpuric meningitis.

44B = history unknown.

at high speed for two minutes in order to throw down aggregates of organisms and particles of culture medium. The supernatant suspension of meningococci was then pipetted into washed cuvettes, placed in a photoreflexometer and diluted with saline solution until a previously determined setting (maintained throughout the experiment) was reached. The resultant suspension was labeled 1. Four dilutions of this were prepared, each one-half the concentration of the preceding; they were labeled respectively 2, 3, 4 and 5. Thus, the concentrations of the materials for inoculation represented one-half, one-fourth, one-eighth and one-sixteenth meningococcal suspension 1.

Groups of 4 rabbits were used for the titration of each strain. The animals were clipped and depilated approximately twenty-four hours before use. Each received two series of five injections, the highest concentration most cephalad, 0.15 cc. being the standard dose. In each animal throughout the entire experiment the left hand series consisted of strain P₁; the right, of the strain being compared to P₁. Twenty-four hours after the preparation of the skin each animal received intravenously 1 cc. of potent supernatant fluid. The prepared sites were examined periodically, most of the lesions appearing within two hours. On one occasion an animal reacted within twenty minutes; frequently the lesions appeared within one hour.

Results.—The results of the titrations carried out on 18 strains of meningococci, 17 associated with known meningococcic purpura or non-purpuric meningitis, are recorded in table 2.

The production of the purpurogenic substance was measured only in terms of strain P_1 . Whether, for example, D_1 is a greater producer than W_1 was not determined. The differences in some instances were small but always definite; when doubt as to classification arose, the strain was considered to be equipotent to P_1 .

Strain 44B was supplied through the kindness of Dr. G. Shwartzman. The potency of its filtrates has been determined by Shwartzman's method through the course of years and found to vary between 600 and 4,000 reacting units per cubic centimeter. Whether it was originally associated with cutaneous purpura is unknown.¹⁶

As seen in table 2, of 8 strains of meningococci isolated from purpuric meningococcemia, 5 fall into the group of greatest producers along with 44B, 2 into the intermediate category, and only 1 into the group of relatively poor producers. Five of the 9 strains from non-purpuric meningitis are equal to P_1 , and 4 are less potent.

All animals were killed within three days after the intravenous inoculation. The organs of those showing gross lesions were sectioned and examined histologically. In both series P_1D_1 and P_1F_1 an animal showed bilateral adrenal hemorrhages. The glands of P_1D_1 were swollen to at least twice normal size and were purple (fig. 4 *A*). The cortices of the organs were seen microscopically to be almost entirely necrotic and hemorrhagic. In a manner completely analogous to bilateral renal cortical necrosis a narrow margin of the zona glomerulosa subjacent to the capsule was intact around the entire periphery of the gland. There were zones of polymorphonuclear leukocytes and erythrocytes demarcating the lesions, many of which showed well advanced ischemic necrosis. The capillaries were only occasionally filled with homogeneous clots, conglutination thrombi. So small was their number that it was impossible to believe that they played a significant role in the production of the lesions. In other sites the damage of the adrenal parenchyma was less marked; here vacuolation of the cytoplasm and early pyknosis of the nuclei testified to the damage already wrought. In both glands the medulla was only moderately involved; some of the veins and arteries were thrombosed.

Study of the adrenal glands of animal P_1F_1 showed a similar but less advanced process. The necrosis again was of the ischemic type, outlined by a zone of hemorrhage. The polymorphonuclear leukocyte response was absent, and only the zona fascicularis was involved. The zona glomerulosa and the zona reticularis, as well as the medulla, were intact. The vessels of the medulla were all patent (fig. 4 *B*).

16. Shwartzman, G.: Personal communication to the authors.

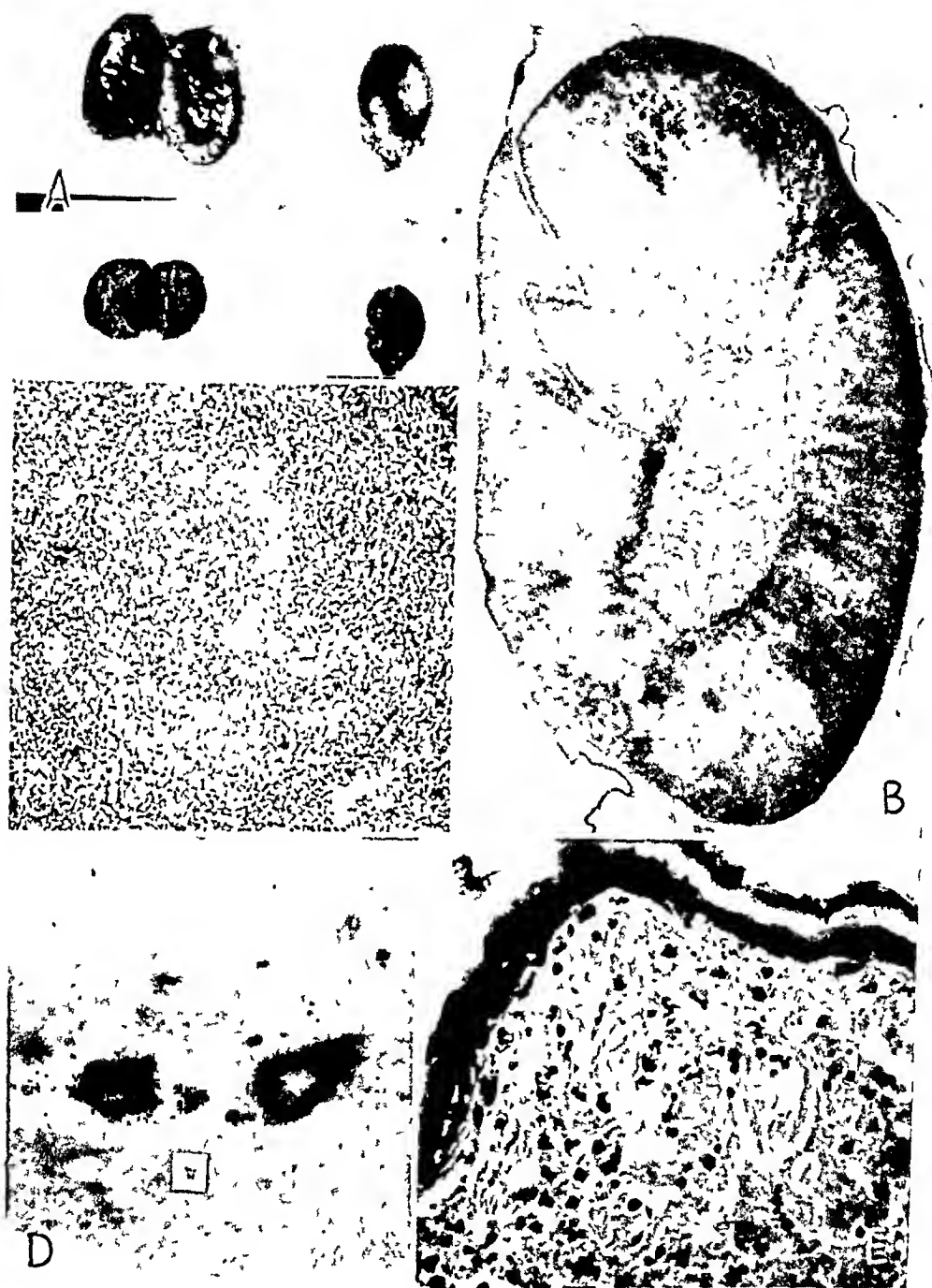


Fig. 4—*A*, necrosis and hemorrhage of adrenal glands accompanying titration of meningococcus strain D_1 against P_1 (experiment 2); the lower pair of glands are normal. *B*, adrenal gland showing necrosis in areas of the cortex (paler areas); $\times 52$. The medulla is intact. (Animal P_1F_1 in experiment 2.) *C*, focus of ischemic necrosis of the spleen (experiment 2); $\times 52$. *D*, Shwartzman reactions at sites of intradermal inoculation of meningococci and cutaneous purpura provoked by meningococcemia (experiment 3). *E*, cutaneous hemorrhage provoked by meningococcemia (experiment 3); $\times 250$.

Aside from small foci of necrosis in the myocardium of P_1D_1 the spleen and the liver revealed the only visible lesions. The splenic sinusoids in both instances were occluded by thrombi which in some areas were so numerous as to result in small foci of infarction (fig. 4 C). The central zones of the hepatic lobules were necrotic; this was especially noticeable in P_1F_1 . The portal fields were not remarkable.

EXPERIMENT 3

Methods.—To test the response of rabbits to sustained meningococemia a saline suspension of twice-washed living meningococci (strain P_1) was prepared as described in experiment 1 and administered intravenously every three hours in doses of 0.5 cc. to groups of 4 rabbits. For the fourth and eighth dose fresh

TABLE 3 (Experiment 3).—Production of Meningococemia and the Related Observations

Meningo-coccus	Rabbit	Intravenous Injections	Local Shwartzman Reaction	Diffuse Cutaneous Purpura	Adrenal Necrosis	Bilateral Renal Cortical Necrosis
P_1	1a*	5	+	+	+	0
	2a	7	0	0	0	0
	3a	7	+	0	0	0
	4a	8	+	+	0	+
P_1	1b	4	0	0	0	0
	2b	6	0	0	0	0
	3b	6	0	0	0	+
	4b	6	+	+	0	0
D_3	1c	3	..	0	0	0
	2c	3	..	0	0	0
	3c	5	..	0	0	+
	4c	6	..	+	0	+
P_1	1d	5	..	0	0	0
	2d	7	..	0	0	+
	3d	7	..	+	0	+
	4d	8	..	0	0	+

* This rabbit presented the Waterhouse-Friderichsen syndrome.

+ The lesion was present.

0 No lesion developed.

material was prepared, and between inoculations the organisms were refrigerated to reduce the danger of contamination.

To provide a field for observation the abdominal hair was clipped and in some groups depilated with barium sulfide approximately twenty-four hours before the experiments were undertaken.

The animals of the first group, labeled "a" in table 3, received two intradermal abdominal depots of the same suspension of P_1 immediately after the first intravenous injection. The animals of group b were treated in an identical manner except that 1 intradermal depot was made rather than 2. Groups c and d received living meningococci intravenously as already outlined without any intradermal depots. Strain D_3 was used instead of P_1 in group c because at the time it was more readily available.

Results.—An unexpected observation was the development of a bleeding tendency in all 16 animals. This tendency became apparent after

the second or third injection as perivenous hemorrhages about inoculation sites and prolonged bleeding time. Hematologic investigations were not undertaken but are to be carried out in the near future.

Animal 1a died some time within the three hours after the fifth inoculation, fifteen to eighteen hours after the experiment was begun. In this period not only did a typical Shwartzman reaction appear at the site of each intradermal depot (fig. 4D) but purpura clearly manifested itself within the depilated abdominal zone. The cutaneous manifestation of meningococcemia was also seen over both flanks and lateral abdominal walls after the hair of the dead animal had been clipped. The petechiae varied from submicroscopic size to about 5 mm. in greatest dimension. The largest were produced in part by confluence of the smaller. They were all purple-red, were not raised above the niveau of the surrounding skin and were particularly numerous on the surfaces of the posterior and anterior flanks.

At necropsy both adrenal glands were at least one and a half times the usual size and flecked with hemorrhage. The remaining organs did not appear remarkable except for occasional small subserosal hemorrhages.

Histologic examination of the heart and the kidneys revealed no recognizable lesions. Some groups of pulmonary alveoli were filled with transudate. The liver was the site of early and definite centrilobular necrosis, and the splenic sinusoids contained occasional thrombi. The adrenal glands showed the same type of focal necrosis with surrounding zones of hemorrhage and well defined areas of early degeneration as were described in experiment 2. The skin presented the only new aspects. In the papillae and the dermis adjacent to the squamous epithelium were foci of hemorrhage (fig. 4E). About some of these were collections of lymphocytes and only few monocytes. The deeper vessels were filled with blood; however, no evidence of thrombosis was visible in any of the sections studied. The zone of Shwartzman reaction showed the changes described in experiment 1.

In animal 3a, as indicated in table 5, only the site of intradermal inoculation reacted in the usual manner. In animal 4a, on the other hand, purpura developed, as well as a localized reaction, similar to but not as marked as that in 1a.

Only animal 4b of group b reacted; in this instance the response was similar to that of 4a, namely, generalized purpura and purpura at the site of intradermal inoculation.

Among the two groups receiving living organisms only intravenously, animals 4c (fig. 5A) and 3d presented the characteristic generalized purpura.

An interesting observation was the appearance of typical bilateral cortical necrosis of the kidneys in 4a, 3b, 3c, 4c, 2d, 3d and 4d (fig. 5B).

The kidneys were examined histologically. In all instances the characteristic necrosis of the glomeruli and the convoluted tubules was seen (fig. 5 *C*). Many of the glomerular capillaries were filled with conglutinated erythrocytes; in 3b fibrin strands were also present. The necrotic tissue was divided into small areas by zones of infiltrating polymorphonuclear leukocytes. These areas, however, were not necessarily separated by intervening segments of preserved kidney. Indeed, the

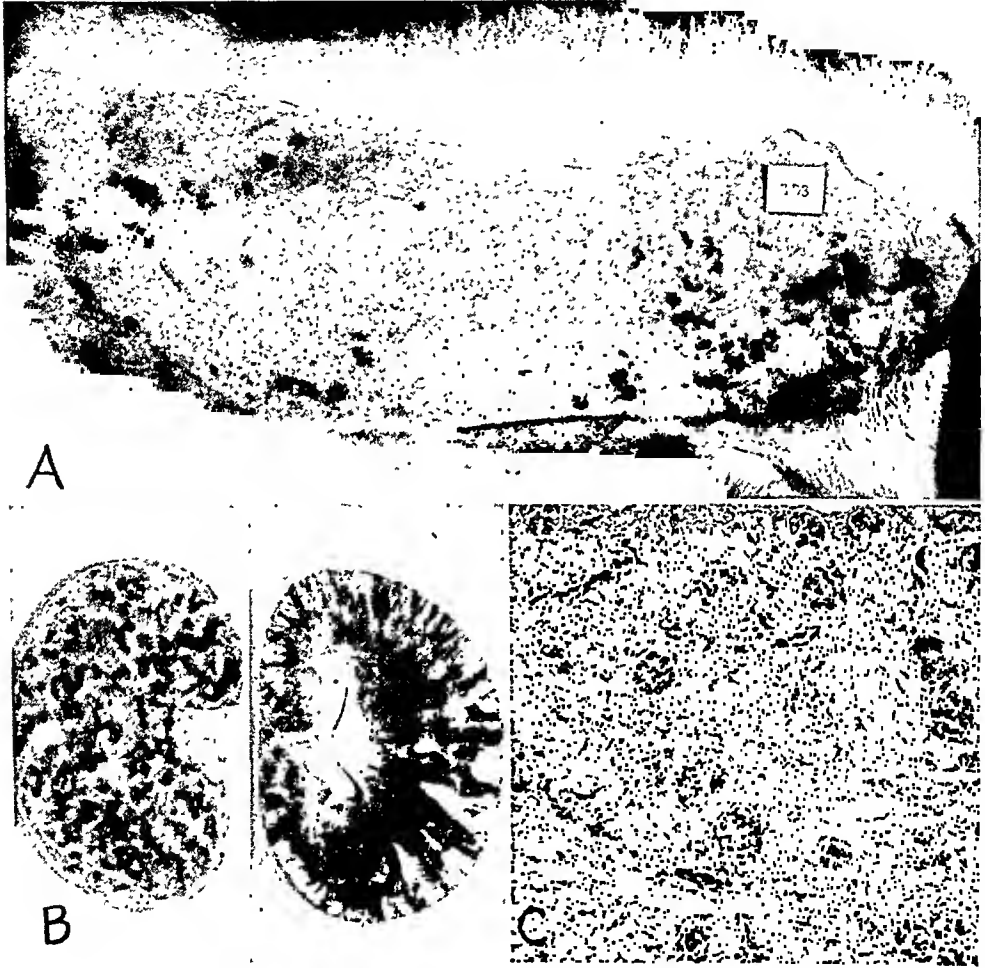


Fig. 5.—*A*, purpuric meningococcemia (experiment 3). *B*, bilateral cortical renal necrosis produced by meningococcemia (experiment 3). In some areas the outer part of the medulla is involved. *C*, necrosis of the renal cortex (experiment 3); $\times 52$.

necrosis sometimes extended almost uninterruptedly over the entire cortex, the older areas of necrosis joined by fresher zones of devastated renal tissue. The arcuate arteries and their larger branches were singularly free from thrombi (fig. 6 *A*). In 3b a few fibrin-containing thrombi were demonstrable (fig. 6 *B*); for the remainder the only evidence of obstruction was conglutinated erythrocytes. In all kidneys

except those of 3d the walls of the interlobular arteries and the afferent arterioles were necrotic. An interesting observation in 4a was necrosis of Henle's loops within the outer zone of the medulla (fig. 5 *B*). The kidneys of animal 3d represented the earliest stage in the evolution of the lesion. The glomeruli and the convoluted tubules were already

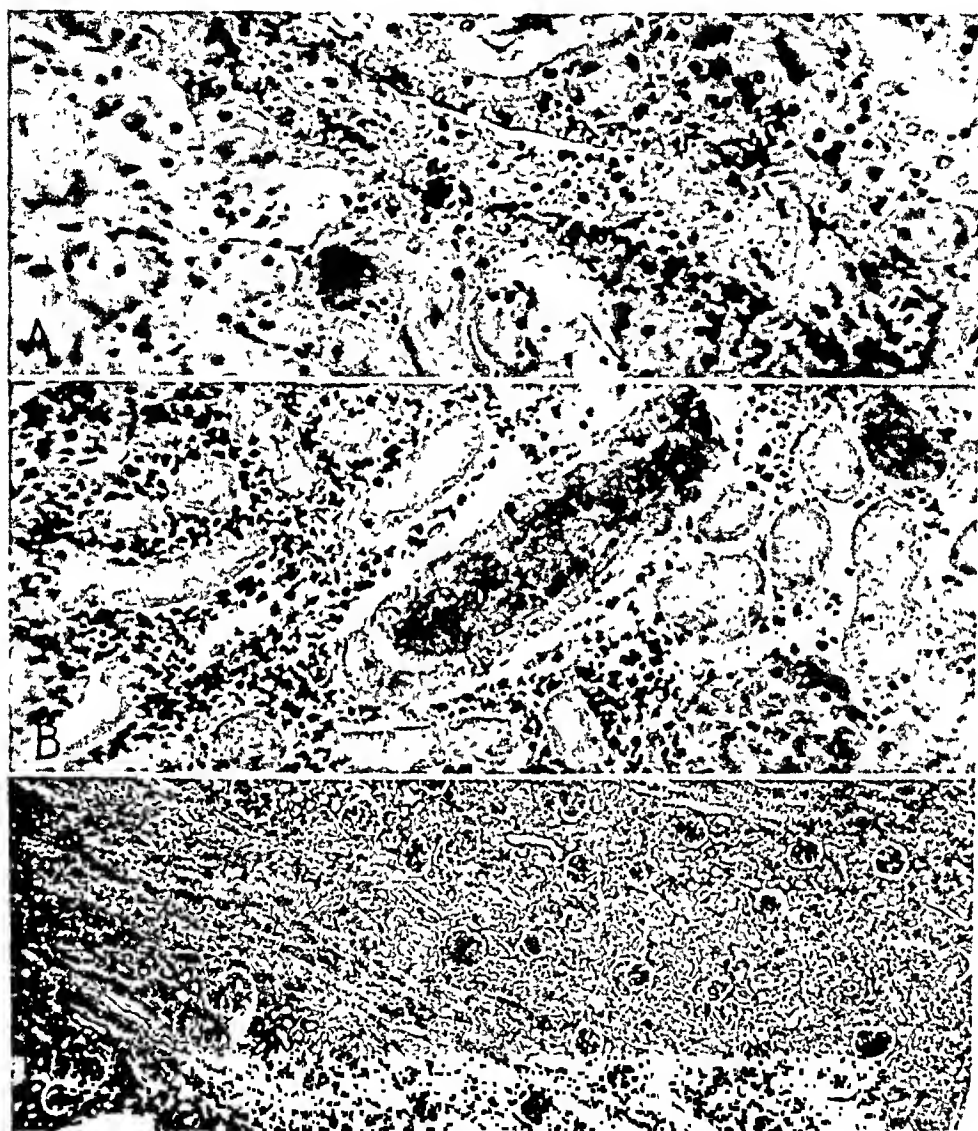


Fig. 6.—*A* and *B*, higher power views of renal cortical necrosis (experiment 3). *A* shows a characteristically patent, although necrotic, interlobular artery; $\times 236$. *B* shows a necrotic interlobular artery filled with thrombus; $\times 236$. *C*, kidney of a killed rabbit showing a focus of nonfatal typical cortical necrosis; $\times 49$.

necrotic, although the structural details were still clearly visible. The leukocytic demarcation line was only barely visible here and there. Conglutination thrombi were seen within capillary lumens in both the

glomerular loops and the cortical interstices. The interlobular arteries and branches were unobstructed and their walls as yet apparently intact.

All histologic preparations of the 4 animals were stained for bacteria by MacCallum's modification of Goodpasture's method¹⁷ and the Brown-Brenn technic.¹⁸ In no instance was it possible to demonstrate the definitive presence of either intracellular or intercellular gram-negative diplococci. It is true that coccoid particles were seen, but because of the large number of pseudoeosinophilic leukocytes and the presence of cellular debris it was impossible to ascertain their identity. Since meningococci have been clearly demonstrated in blood smears from cutaneous purpura of meningococcemia stained by Gram's method, the absence of organisms in the experimental lesions was investigated. For this purpose 2 rabbits received a number of intradermal inoculations, each consisting of 0.15 cc. of a heavy suspension of gram-negative diplococci (*G*₂). The lesions following the inoculations were removed under ether anesthesia at the following intervals: one hour and fifteen minutes, six hours and thirty minutes, ten hours, fifteen hours and twenty-four hours and thirty minutes. Each specimen was fixed in Zenker's fluid, and sections were stained with simple methylene blue and by MacCollum's modification of Goodpasture's method, the Brown-Brenn method and the Glynn modification of Gram's method.¹⁷ In the methylene blue preparation of the first specimen masses of recognizable diplococci were seen. MacCollum's and Glynn's stains showed them as faintly gram-negative particles, recognizable as meningococci only because of the knowledge of their location gained from studying the first section. In the Brown-Brenn preparation the organisms could not be seen. The second specimen removed revealed in the MacCollum, Glynn and Brown-Brenn stained sections no identifiable gram-negative diplococci. Meningococci were, however, presumed to be recognized in the methylene blue preparation.

The same results were found in the remaining three specimens. With the progressive accumulation of leukocytes and the appearance of cellular detritus, organisms were even more difficult to identify.

This signal failure to differentiate meningococci in rabbit tissue by means of the usual tinctorial methods after large numbers of demonstrable gram-negative diplococci were inoculated intradermally is a pregnant comment on the difficulty of staining, as well as of the recognition of, *Neisseria* in fixed tissues. Push's report¹⁹ exemplifies this difficulty in contrast to the easy success which others have reported.

17. Mallory, F. B.: *Pathological Technique*, Philadelphia, W. B. Saunders Company, 1938.

18. Schaub, I. G., and Foley, M. K.: *Methods for Diagnostic Bacteriology*, St. Louis, C. V. Mosby Company, 1943.

19. Thomas, H. B., and Leiphart, C. D.: *J. A. M. A.* **125**:884, 1945.

One animal from each of groups a and c showed the same type of pulmonary arteritis described in rabbit CE₄ of experiment 2.

MENINGOCOCCIC PURPURA

In the past relatively few attempts have been made to test the ability of living or dead bacteria to produce the Shwartzman phenomenon. The work of several authors²⁰ employing unwashed cultures may not be considered, since the inoculums may have contained besides the organisms relatively large amounts of soluble potent substances.

Witebsky and Salm,²¹ working with washed suspensions of *Haemophilus influenzae*, Moritz¹² with *Salmonella aertrycke* and Witebsky and Neter²² with *Pneumococcus* were able to demonstrate the ability of their respective bacteria to prepare the skin and/or elicit the phenomenon. *Pneumococcus* possessed only the power to prepare skin.

Two groups of investigators employed meningococci in their experiments. Riley and Wilson²³ in their study of the toxicity of various meningococcic preparations successfully used heat-killed washed meningococci to prepare the site and evoke the Shwartzman phenomenon. Schneirerson²⁴ prepared the skin of rabbits with living washed, as well as dead dried, meningococci. He found that while the living cocci could provoke the reaction the heat-killed meningococci could not.

It appears, then, that the experiments reported in table 1 confirm in full the work of Riley and Wilson and of Schneirerson, except for the latter's inability to provoke the phenomenon with dead dried organisms. Since Schneirerson heated the meningococci for one hour at 56 C., in contrast to our fifteen minutes at 60 C., his failure may have been due to heat inactivation of the potent principle, despite its relative heat stability.

The design of experiment 1 is such as to make possible a critical statement concerning Shwartzman's assertion⁶ that the filtrate of a given culture contains much more of the potent materials than may be found in the disintegrate of its organisms. In our hands the washed meningococci, both living and dead, are far more potent than the supernatant, which corresponds to Shwartzman's filtrate.

Assuming that the 18 strains are a representative sample of all pathogenic meningococci, simple inspection of table 2 makes it apparent

20. Sanarelli, G.: *Ann. Inst. Pasteur* **38**:11, 1924. Gratia, A., and Linz, R.: *Compt. rend. Soc. de biol.* **107**:1579, 1931. Koplik, L. H.: *Am. J. Path.* **11**:842, 1935. Alechinsky, A., and Renaux, E.: *Compt. rend. Soc. de biol.* **128**:790, 1938. Alechinsky, A.: *ibid.* **129**:513, 1938. Shwartzman.⁶

21. Witebsky, E., and Salm, H.: *Proc. Soc. Exper. Biol. & Med.* **34**:351, 1936.

22. Witebsky, E., and Neter, E.: *Proc. Soc. Exper. Biol. & Med.* **38**:187, 1938.

23. Riley, C. V., and Wilson, M. A.: *J. Immunol.* **23**:269, 1932.

24. Schneirerson, S. S.: *J. Infect. Dis.* **65**:97, 1939.

that all strains of *Neisseria meningitidis* causing human disease are potentially capable of eliciting cutaneous purpura since in all three groups such organisms are present.

It is believed that the purpurogenic substance is identical in all strains, varying only quantitatively among the organisms of the three categories of table 2. Since in the first column 5 strains are associated with purpura and none of the 9 nonpurpuric strains falls into this group, it appeared desirable to recast the results in the form of a contingency table by combining the central and right columns of table 2. Of 5 strains with a potency greater than P_1 , all were from instances of purpuric meningococcemia. The potency of none of the 9 strains isolated from cases of nonpurpuric meningitis was greater than that of P_1 . The probability of such a distribution being a matter of chance is 8 per cent (restricted point binomial method). While this result does not establish a statistically significant distribution, the trend in this small group is such as to make inescapable the conclusion that the presence or the absence of cutaneous purpura in meningococcic disease is in part dictated by the strain of *Neisseria* causing the infection.

That this factor, however, is not solely responsible for purpuric meningococcemia is evident. P_1 , F_1 and D_1 , table 2, while associated with cutaneous purpura, are nevertheless equipotent only with the 9 nonpurpuric strains. It follows, therefore, that in some instances a factor or factors other than the purpurogenic substance play a decisive role.

Since meningococci may be divided into four serologic groups, the possible relationship of these innate differences to the presence or the absence of purpura in the human sources of the organisms was investigated. Table 2 clearly shows the absence of any such relationship. However, an observation made in all three experiments, embracing a total of 125 rabbits, appears pertinent to the discussion, namely, the factor of individual reactivity. As may be seen by examination of tables 1 and 3, while some animals react, others do not to the almost simultaneous inoculation of living or dead meningococci prepared at the same time and obtained from the same source. Because of this, it was impossible to predict in detail the outcome of any particular experiment.

The assay of strain F_1 against P_1 , stylized in figure 7, illustrates this factor. Rabbit 1 did not react in any of the prepared sites. In contrast, animal 2 responded in all P_1 and four of the F_1 sites. Animals 3 and 4 show reverse images of their reactions, animal 3 reacting in the first two and three sites of F_1 and P_1 , respectively, and animal 4 in the first three and two sites of F_1 and P_1 .

Drawing, therefore, an analogy between the rabbits and man, it may be said that meningococcic purpura is most apt to appear through

the fortuitous infection of a person with marked susceptibility to the Shwartzman toxin by a very purpurogenic meningococcus.

The individual variability and perhaps the meningococcus strain likewise appear to determine the time required for reaction following the provocative inoculation. In the assay of P_3 1 animal responded in all ten sites twenty minutes after the intravenous injection. Many animals showed reactions after one hour; others, only after two or three hours.

Experiment 3 is the logical sequel to experiments 1 and 2 and represents an attempt to produce generalized meningococcic purpura in a manner similar to that in human subjects. Not recorded in table 3 is the fact that the Shwartzman reactions at the sites of intradermal inoculation in animals 1a (fig. 4D), 4a and 4b coincided with the

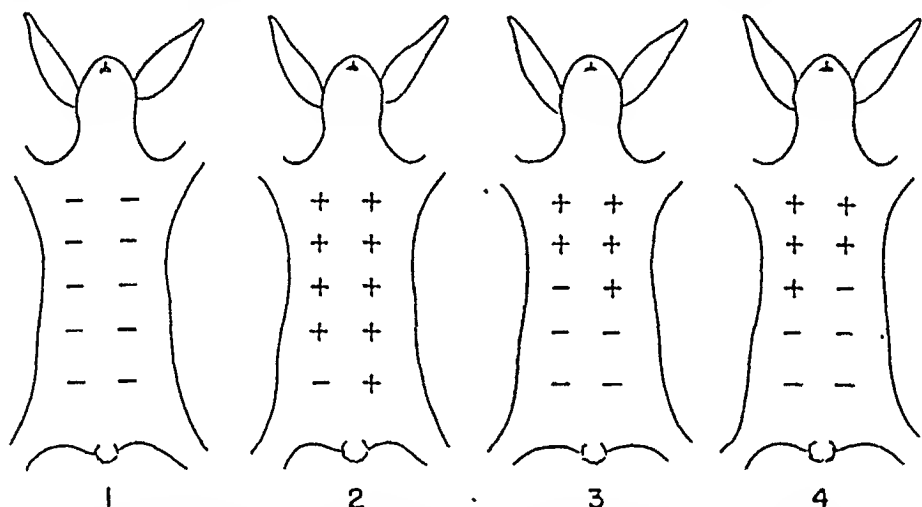


Fig. 7.—Stylization of the titration of the preparatory potency of meningococcus strain F_1 (left) against that of strain P_1 (right) (experiment 2).

appearance of the general cutaneous purpura. The converse of the foregoing statement is likewise significant, namely, that in no instance did general purpura appear without a corresponding reaction in the intradermal depot. These observations strongly suggest that genetically both lesions are closely related, if not identical.

Animal 3a, which had a weakly positive Shwartzman reaction about the intradermally deposited meningococci, did not suffer general purpura despite its seven intravenous inoculations of living cocci. Since the extent of a local Shwartzman reaction in a given animal is directly related to the amount of potent material inoculated, it follows that of the two (local reaction about the depot and general purpura) the former is more apt to occur, since more meningococci have been inoculated than could possibly reach an equal area of skin through the blood stream.

The c and d series of rabbits received only intravenous inoculations of living organisms, and characteristic cutaneous purpura (fig. 5A)

developed in 2 rabbits, 4c and 3d, confirming the results of groups a and b.

If it is assumed, therefore, that each cutaneous lesion represents a Schwartzman reaction in miniature, the following interpretation of meningococcic purpura appears warranted. The human disease is most frequently preceded by an infection of the upper respiratory tract, from which locus, it is believed, meningococci enter the blood stream and multiply. Neisseria and their products are deposited in the skin and prepare it. The time necessary to achieve this varies with the patient's reactivity and the strain of the infecting meningococcus. When the incubation time is spent, the patient, displaying meanwhile the symptoms of continuing bacteremia, suddenly presents the first purpuric lesions. These are produced by the provocatory effect of the bacteremia. The number and the size of the lesions depend again on the two factors just mentioned: the patient's reactivity and the potency of the infecting strain of meningococcus. Thus, in some subjects, human and rabbit, no lesions develop; in others a few petechias, and in still others massive confluent purpuric areas. The continuing appearance of the lesions is due to the further provocation of foci established later in the course of the bacteremia.

It is understood that the foregoing statement represents a working hypothesis which remains to be confirmed by a study of the effects of meningococcemia on the constituents of blood and ultimately the isolation of the factor or factors responsible for the reaction.

THE WATERHOUSE-FRIDERICHSEN SYNDROME

The one microscopic and three macroscopic instances of adrenal necrosis and hemorrhage of experiments 2 and 3 raise the problem of the Waterhouse-Friderichsen syndrome.

It is well known that necrosis of the adrenal glands associated with varying degrees of hemorrhage may occur not only in a large number of infectious diseases (meningococcemia, diphtheria,²⁵ influenza²⁶ and others) but also in noninfectious states (toxemia of pregnancy,²⁷ severe burns,²⁸ as well as in the newborn²⁹) and a host of more or less poorly understood conditions.³⁰ Experimental procedures of various types have likewise resulted in the production of adrenal necrosis and hemor-

25. MacLean, A.: *J. Hyg.* **37**:345, 1937.

26. Lucké, B.; Wight, T., and Kime, E.: *Arch. Int. Med.* **24**:154, 1919.

27. Dodds, G. H.: *Brit. M. J.* **1**:769, 1945

28. Harkins, H. N.: *The Treatment of Burns*, Springfield, Ill., Charles C Thomas, Publisher, 1942, chap. 6.

29. Goldzieher, M. A., and Murray, G. B.: *Endocrinology* **165**:181, 1932.

30. Mitchell, W., and Angrist, A.: *Am. J. M. Sc.* **205**:549, 1943.

rhage; administration of drugs,³¹ bacteria and their products,³² dietary deficiencies³³ and various forms of acute shock³⁴ are but a few of the methods utilized.

In all of the more authoritative reports the adrenal necrosis and hemorrhage have been in the zona fasciculata and to a lesser extent in the zonae glomerulosa and reticularis. The medulla is usually preserved. The descriptions of the glands, regardless of etiologic agent, correspond to that recorded in the protocols of this report. Whether a common factor underlies the common end result is a moot question. Hoerr³⁵ suggested, on the basis of Neuman's work,³⁶ that since the adrenal glands possess the greatest blood supply of all organs they must likewise receive a corresponding share of any circulating noxious material and are therefore more liable to injury. On the other hand, one must consider that an abundant circulation may be necessary to the survival of the adrenal parenchyma. If this is the case, moderate impairment of the general circulation may reflect itself in decreased adrenal function and severe impairment, perhaps in necrosis. Thus may be explained the adrenal necrosis of acute experimental hemorrhagic and dehydration shock. It appears therefore that pathologic states combining both possibilities—circulating toxic substances and circulatory collapse—should be more frequently associated with severe adrenal cortical damage. Human meningococcic disease may represent such a condition.

The incidence of purpuric meningococcemia varies from epidemic to epidemic, sometimes rising as high as 50 per cent.³⁷ In only a small number of the purpuric patients does adrenal necrosis develop. In analogy, only 2 of the 5 animals of table 3 with cutaneous purpura showed adrenal changes, 1a marked changes, 4a one microscopic focus. These results support the view that purpuric meningococcemia is not synonymous with the Waterhouse-Friderichsen syndrome, of which adrenal necrosis and hemorrhage are an integral part.

If meningococcemia is a disease which disposes toward adrenal damage, why do so few subjects show widespread adrenal cortical

31. (a) Humphreys, E. M., and Donaldson L.: *Am. J. Path.* **17**:767, 1941. (b) Reyna, F. G.: *Beitr. z. path. Anat. u. z. allg. Path.* **97**:268, 1936. (c) Flexner, S.: *J. Exper. Med.* **2**:197, 1897.

32. Olitsky, L.; Avinery, S., and Koch, P. K.: *J. Immunol.* **45**:237, 1942. Oppenheim, R., and Loeper, M.: *Arch. de méd. expér. et d'anat. path.* **13**:332, 1901. Gerber.¹⁶

33. Supplee, G. C.; Bender, R. C., and Kahlenberg, O. J.: *Endocrinology* **30**:355, 1942.

34. Davis, H. A.: *Arch. Surg.* **47**:939, 1941. Muirhead, E. E.; Ashworth, C. T.; Kregel, L. A., and Hill, J. M.: *ibid.* **45**:863, 1942.

35. Hoerr, N.: *Am. J. Anat.* **48**:139, 1931.

36. Neuman, K. O.: *J. Physiol.* **45**:188, 1912-1913.

37. Herrick, W. W.: *J. A. M. A.* **71**:612, 1918.

necrosis? This question suggests a third possible cause of the adrenal lesions.

Gerber¹⁵ observed that a single intravenous inoculation of potent bacterial filtrate may produce unilateral adrenal lesions such as were encountered in the present series. Since it has been confirmed by thousands of experiments that the Shwartzman phenomenon may be produced only by first preparing tissue and then provoking the response via an intravenous inoculation, the adrenal necrosis may not be considered part of the Shwartzman phenomenon. The low incidence (2.4 per cent in 125 animals) of this series supports this point of view. The work of Gratia and Linz,³⁸ soon confirmed by Shwartzman and Michailovsky,³⁹ demonstrated the ability of one intravenous injection of potent filtrate to produce hemorrhagic necrosis of certain tumors. No satisfactory explanation has been forthcoming in elucidation of this remarkable phenomenon. The inference cannot be avoided that in some mammals, human and rabbit, the relationship of adrenal vasculature to adrenal parenchyma and the sensitivity of the structures to the Shwartzman toxin may be of the same order as that existing between the toxin and these tumors.

Since the adrenal lesion is an intrinsic part of the Waterhouse-Friderichsen syndrome but apparently not a result of the mechanism producing the cutaneous purpura (Shwartzman phenomenon), it appears fallacious to write of curing the syndrome, as so many have, when what is meant is the successful treatment of petechial meningococcemia. Until clinical means are devised for differentiating impairment of adrenal function from adrenal necrosis, definitive diagnosis of the Waterhouse-Friderichsen syndrome must remain within the province of the pathologist.

In support of their belief that the destruction of the adrenal cortex in meningococcemia is just another hemorrhage in a hemorrhagic disease and without significant effect on its further course, Thomas and Leiphart¹⁰ pointed out that septicemic shock and death may occur without histologic evidence of widespread adrenal damage. Furthermore, removal of dogs' and rats' adrenal glands does not immediately result in death of the animals but is followed by a gradual decline over a period of days. While both statements are true, it does not appear reasonable to compare the result of adrenalectomy in a healthy animal with that of spontaneous destruction of the adrenal cortices in a desperately sick person. It would appear better judgment to assume that such a lesion would hasten, if not precipitate, the fatal termination of the illness.

38. Gratia, A., and Linz, R.: *Compt. rend. Soc. de biol.* **108**:427, 1931.

39. Shwartzman, G., and Michailovsky, N.: *Proc. Soc. Exper. Biol. & Med.* **29**:737, 1932.

During the increase of meningococcic disease associated with World War II several authors⁴⁰ have stated more or less categorically that the sole cause of the Waterhouse-Friderichsen syndrome is meningococcemia. That such statements are intemperate is evident to students of the syndrome. In most recorded cases the bacteriologic studies made have been inadequate or unsuccessful, and in a small number of instances organisms other than meningococci have been demonstrated. One of us (Dr. Black-Schaffer) has made postmortem examinations of a number of persons with this syndrome. In 2 the syndrome undoubtedly was caused by, respectively, beta hemolytic streptococci and pneumococci.⁴¹ Since the Schwartzman substance is not confined to the meningococcus but is found, albeit in lesser concentration, in other organisms, the occasional production of the syndrome by bacteria other than meningococci is not surprising.

BILATERAL RENAL CORTICAL NECROSIS

The bilateral cortical necrosis of the kidneys appearing in animals of experiment 3 was not entirely unexpected. Apitz¹³ produced the lesion with two intravenous inoculations of potent bacterial filtrate, given twenty-four hours apart. This has since come to be recognized as characteristic of the generalized Schwartzman reaction. Since the living and dead washed meningococci have thus far been shown to possess properties similar to those of the corresponding bacterial filtrates and supernatant fluids, there was every reason to believe that an experiment reproducing the essentials of Apitz' work would achieve the same result: bilateral renal cortical necrosis.

In numerous experiments Apitz,¹³ Gerber¹⁵ and others have proved (with one exception to be discussed later) that the renal changes cannot be elicited, as can the local cutaneous reaction after suitable preparation, by a single intravenous inoculation of the Schwartzman substance.

Since washed living meningococci are able to produce this remarkable lesion when inoculated every three hours for a period of fifteen to twenty-four hours, the conclusion drawn from experiment 1—that meningococci possess the same properties as their filtrates—is buttressed. The concept of the mechanism of experimental meningococcic purpura and, by analogy, the human disease, first suggested by Schwartzman⁶ and developed in this paper, is likewise reenforced. It clearly demonstrates the ability of the organisms of a continuous meningococcemia to prepare tissues and provoke the typical response, cutaneous purpura and/or bilateral renal cortical necrosis.

40. Martland, H. S.: *Arch. Path.* 37:147, 1944. Herbut and Nanges.^{1c} Thomas and Leiphart.¹⁹

41. Black-Schaffer, B.: Unpublished data.

Duff and Murray⁴² in their otherwise excellent review of the subject of bilateral renal cortical necrosis unfortunately overlooked the generalized Shwartzman reaction. They reached the conclusion that the pathogenesis of the renal changes is intense vasospasm or vaso-paralysis followed by necrosis of the interlobular arteries and their afferent arterioles, with accompanying stasis of circulation, possibly thrombus formation in some cases, and consequent ischemic necrosis of the renal cortex.

The lesions in the animals of experiment 3 were identical with those of symmetric renal cortical necrosis in man and animals, regardless of cause.

The pathologic anatomy of the kidneys studied in this series lends support to the hypothesis likewise supported by the work of Byrom,⁴³ deNavasque⁴⁴ and Penner and Bernheim.⁴⁵ The relative infrequency of other than conglutination thrombi and the necrosis of the arterioles and the interlobular arteries observed in the cases with older lesions fit well into the concept of primary vascular damage leading to circulatory stasis with subsequent conglutination of stagnant erythrocytes. In a preliminary report of recent date, Trueta and his colleagues⁴⁶ imparted the information that they had experimentally demonstrated a mechanism whereby the renal circulation is shunted directly from the larger arteries through the vasa rectae to the medullary veins, producing cortical ischemia. The mechanism is under neural control and may be elicited by a number of stimuli. They suggested that this shunt may play a considerable role in the production of the oliguria or anuria of trauma, puerperal accidents and other incidents. Such evidence, although not yet confirmed, pertinently supports the school of thought which insists on vasospasm as the cause of renal cortical necrosis.

A factor in our animals which at the present time cannot be adequately evaluated is the hemorrhagic diathesis previously mentioned but not discussed. The experimental production of necrosis of the renal cortices in 7 of 16 animals (experiment 3), all showing a bleeding tendency, favors the point of view that thrombosis of the renal vasculature may not be an important primary mechanism in the genesis of the condition.

The experimental and known toxicologic methods of producing bilateral cortical necrosis of the kidneys are varied. Intravenously

42. Duff, G. L., and Murray, E. G. D.: *Am. J. M. Sc.* **201**:428, 1941.

43. Byrom, F. B.: *J. Path. & Bact.* **45**:1, 1937.

44. deNavasque, S.: *J. Path. & Bact.* **46**:47, 1938.

45. Penner, A., and Bernheim, A. L.: *Arch. Path.* **30**:465, 1940.

46. Trueta, J.; Barclay, A. E.; Franklin, K. J.; Daniel, P., and Prichard, M. M. L.: *Lancet* **2**:237, 1946.

injected staphylococcic filtrates⁴⁷ have been employed to effect the changes in rabbits and cats. Repeated intravenous injections of hog cholera virus vaccine⁴⁸ likewise resulted in the appearance of the typical lesion. Simple chemical agents (intravenous lithium carmine in rabbits⁴⁹ and dioxane,⁴⁹ diethylene glycol⁵⁰ and almond extract⁵¹ poisoning in man) have also produced the renal lesion. The generalized Schwartzman reaction is part and parcel of this heterogeneous group of etiologic agents, whose mode of action is essentially unknown. In another category are the results of two experiments employing known vasospastic drugs: pitressin in rats⁴³ and epinephrine hydrochloride in dogs.⁴⁵ In these instances the characteristic lesion is ascribed to vasoconstriction with necrosis of the afferent arterioles and ischemia of the involved nephrons. Byrom⁴³ found, when large doses of pitressin were used, that the vascular necrosis extended to and involved the larger vessels of the kidneys. Penner and Bernheim⁴⁵ examined the literature of renal cortical necrosis and reviewed those striking cases in which a direct relationship appears to exist between traumatic or surgical shock and the onset of fatal urinary suppression in males and nonpregnant females. They concluded that in some human subjects the vasospasm of shock is sufficient to result in bilateral renal cortical necrosis.

The occurrence of the lesion in men and nonpregnant women is being reported more frequently.⁴² It is now known that it may have a variety of initiating causes. Acute infections compose the largest group in this category. The results of our experiments do not permit conclusions on the relationship between acute infection and bilateral renal cortical necrosis. However, Gerber's cautious statement⁴⁵ may be reaffirmed: ". . . the changes observed in spontaneous and experimental infections in animals and in association with infection in man suggests that a similar pathogenesis may be operative." It is now demonstrated that not only extreme artefacts, such as those produced in the experiments of Apitz,⁴³ deNavasque,⁴⁴ Rigdon and co-workers^{47a} and others, but a condition more closely approximating a natural infection, meningococcemia, is capable of eliciting the same results. In fact, the meningococcemia appears to be at least as effective in producing the changes as the inoculation of bacterial filtrates.

No report of bilateral renal cortical necrosis complicating meningococcic disease has appeared in the literature. This seems at variance

47. (a) Rigdon, R. H.; Joyner, A. L., and Ricketts, E. T.: *Am. J. Path.* **10**:425, 1934. (b) Von Glahn, W. C., and Wild, J. T.: *J. Exper. Med.* **61**:1. 1935. deNavasque.⁴⁴

48. Röhrer, H.: *Virchows Arch. f. path. Anat.* **284**:203, 1932.

49. Barber, H.: *Guy's Hosp. Rep.* **84**:267, 1934.

50. Geiling, E. M. K., and Cannon, P. R.: *J. A. M. A.* **111**:919, 1938.

51. Garvin, C. F., and van Wezel, N.: *Arch. Int. Med.* **62**:423, 1938.

with the expectations of the preceding exposition and discussion. However, the extreme rarity of the condition in contrast to the everyday occurrence of all kinds of infections, the ability not only of meningococcus but of staphylococcal toxins and hog cholera virus to produce the lesion, and the complete absence of correlation of any particular infection with renal cortical necrosis make it abundantly evident that infection is of secondary importance in its genesis. These facts have led Duff and Murray⁴² to assume that in certain apparently normal persons the interlobular arteries and glomerular arterioles may be hyperirritable.

The one exception mentioned in the foregoing discussion to the rule that renal cortical necrosis may not be produced with a single intravenous injection of Shwartzman filtrate is pregnancy.⁴³ Therefore, the fact that the most common etiologic moment in man is likewise pregnancy is of more than passing interest.

Three instances of postpartum necrosis of the renal cortices studied by one of us⁴² revealed an important anamnestic factor: prepartum or intrapartum hemorrhagic shock associated with ablatio placentae. Perusal of the literature brings this dramatic prologue to the onset of uremia into the foreground. In 29⁴⁴ of 50 reported cases of bilateral necrosis of the renal cortices complicating delivery this condition was preceded or accompanied by severe hemorrhage. The histories of 4 additional cases⁴⁴ were recorded in such a manner as to justify the interpretation that marked prepartum or intrapartum hemorrhage was present. In 1 other instance,⁴⁵ while hemorrhage is not mentioned, profound shock

52. Black-Schaffer, B.: Unpublished data.

53. (a) Bradford, J. R., and Lawrence, T. W. P.: *J. Path. & Bact.* **5**:189, 1898. (b) Cruickshank, J. N.: *J. Obst. & Gynaec. Brit. Emp.* **30**:336, 1923. (c) Dalrymple, S. C.: *New England J. Med.* **203**:160, 1930. (d) Davis, C. H.: *J. A. M. A.* **114**:2370, 1940. (e) Geipel, P.: *Zentralbl. f. Gynäk.* **38**:517, 1914. (f) Glynn, E. E., and Briggs, H.: *J. Path. & Bact.* **19**:321, 1914-1915. (g) Griffith, W. S. A., and Herringham, W. P.: *ibid.* **11**:237, 1906-1907. (h) Jardine, R., and Kennedy, A. M.: *Lancet* **1**:1291, 1913 (case 1). (i) Lloyd, H. C.: *ibid.* **1**:156, 1906. (j) Manley, J. R., and Kliman, F. E.: *Am. J. Obst. & Gynec.* **14**:802, 1927. (k) Reyna, F. G.: *Beitr. z. path. Anat. u. z. allg. Path.* **97**:268, 1936. (l) Scriber, W. deM., and Oertel, H.: *J. Path. & Bact.* **33**:1071, 1930. (m) Sheldon, W. H., and Hertig, A. T.: *Arch. Path.* **34**:866, 1942 (2 cases). (n) Stening, M. J. L.: *J. Obst. & Gynaec. Brit. Emp.* **46**:250, 1939. (o) Warner, C. G., and Hibbits, J. T.: *Am. J. Obst. & Gynec.* **23**:875, 1935. (p) Westman, A.: *Acta obst. et gynec.* **7**:235, 1928 (case 1). (q) Tomlinson, W. J.: *Am. J. Obst. & Gynec.* **49**:236, 1945. (r) Davidson, J., and Turner, R. L.: *Tr. Edinburgh Obst. Soc.* **89**:101, 1929-1931 (cases 1 and 3). (s) Dunn, J. S., and Montgomery, G. S.: *J. Path. & Bact.* **52**:1, 1941 (6 cases). (t) Kellar, R. J., and Arnott, W. M.: *Tr. Edinburgh Obst. Soc.* **92-94**:101, 1932-1935 (cases 2 and 3).

54. Evans, N., and Gilbert, E. W.: *Am. J. Path.* **12**:553, 1936. Rolleston, H. D.: *Lancet* **2**:1173, 1913. Schüppel, A.: *Arch. f. Gynäk.* **103**:243, 1914. Torrens, J. A.: *Lancet* **1**:99, 1911.

55. Jardine, R., and Teacher, J. H.: *J. Path. & Bact.* **15**:137, 1910-1911 (case 2).

is said to have been evident. In 16 cases⁵⁶ hemorrhage or shock is said to have been absent or is not mentioned. Thus, of 53 women, 37 (69.8 per cent) were definitely the victims of severe shock, hemorrhagic or other, promptly followed by oliguria and uremia. It is impossible to estimate the real incidence, since most early authors regarded hemorrhagic shock as an unimportant, even though serious, complication. It is evident, therefore, that this figure represents a minimum incidence.

Many of the hemorrhages were of the ablatio (abruptio) placentae type. All textbooks of obstetrics are unanimous in asserting that the most important and constant symptom of this feared accident is profound shock, frequently entirely out of proportion to the blood lost. It is particularly in this type of obstetric accident that urinary suppression, with or without recovery, is most frequently encountered.

In modern times oxytocic substances, usually a pituitary extract or an ergot preparation, are used, either singly or together, in routine postpartum therapy. Two factors which are known vasospastic agents were thus present in most of the cases: shock and an oxytocic extract or drug. It is not improbable that the effect of this set of circumstances acting on a vasculature morbidly affected by pregnancy results in spasm of the small arteries and arterioles of the kidneys. The vascular disturbance is evident in the fact that the frequency of ablatio placentae is four times greater in cases of toxemia of pregnancy than in cases of apparently normal gravidity.⁵⁷ The spasm may be of sufficient intensity and duration to result in damage of the renal parenchyma, in some instances irreversible, in others transient, manifested by oliguria and uremia. In support of this hypothesis are reports of recovery, substantiated in 1 important instance by biopsy⁵⁸ of a woman's kidney after postpartum anuria developed. Figure 6C is the photomicrograph of the kidney of a rabbit killed forty-eight hours after intravenous inoculation of potent meningococcic filtrate, showing a small focus of typical cortical necrosis. The glomerulus and its convoluted tubules are necrotic. Similar small lesions are scattered throughout both renal

56. (a) Bowers, R. K.: *Proc. Roy. Soc. Med.* 27:1505, 1934. (b) Geipel, P.: *Arch. f. Gynäk.* 124:231, 1925. (c) Heist, J. C.: *Am. J. Obst. & Gynec.* 12:673, 1926. (d) Herzog, G.: *Beitr. z. path. Anat. u. z. allg. Path.* 56:175, 1913. (e) Immink, E. A.: *Nederl. tijdschr. v. geneesk.* 74:2389, 1930. (f) Klotz, O.: *Am. J. Obst.* 58:619, 1908. (g) Zooijer, G. H.: *Mitt. a. d. Grenzgeb. d. Med. u. Chir.* 12:754, 1903. (h) Carson, W. J., and Rockwood, R.: *Arch. Path.* 1:889, 1926. (i) Bruno, F. E.: *New Orleans M. & S. J.* 94:596, 1942. (j) Jardine and Kennedy.^{53b} (k) Westman.^{53p} (l) Davidson and Turner.^{53r} (m) Dunn and Montgomery.^{53s} (n) Kellar and Arnott.^{53t} (o) Jardine and Teacher.⁵⁵

57. Beck, A. C.: *Obstetrical Practice*, ed. 3, Baltimore, Williams & Wilkins Company, 1942, p. 706.

58. Crook, A.: *Proc. Roy. Soc. Med.* 20:1249, 1927.

cortices, with normal-appearing tissue intervening. In appearance and distribution these lesions are identical with those illustrated in the drawing and photomicrographs of Crook's report.⁵⁵ In contrast, another animal in this group of 3 died twenty hours earlier, but its kidneys already showed the characteristic appearance of advanced cortical necrosis. The third succumbed six hours after the provocative injection, too soon for lesions to become visible. Such striking differences, also encountered by deNavasque in his experimental work, indicate that there is little reason to believe that in human as well as in experimental bilateral renal cortical necrosis an "all or none" law prevails, as some authors discussing reports of recovery would have their readers infer.

It appears from this discussion that in man numerous agents are capable of releasing a sequence of events leading to bilateral renal cortical necrosis. The most common cause is shock in parturient women. The same etiologic factor in males and nonpregnant females has been inculcated by numerous observers and confirmed to some extent by experiments utilizing known vasospastic substances.

An interpretation of the genesis of the renal lesion produced by both the generalized Shwartzman phenomenon and experimental meningococcemia based on the facts stated in the foregoing pages is as follows: The preparation of the kidney consists in the establishment of a state of vascular hyperirritability: the provocation, in a spasm of these vessels resulting in circulatory stasis, ischemia of the cortex and conglutination of the stagnant erythrocytes. Should the spasm continue beyond a certain time, necrosis of parenchyma and vessels occurs.

Several authors have reported the presence of varying degrees of necrosis of the liver, the adrenal glands, the spleen, the pituitary gland and the gastrointestinal tract in various combinations accompanying human renal cortical necrosis. Since the hypotheses examined in relation to the adrenal and renal lesions need no refurbishing, little is to be gained by an exhaustive review of these cases. It appears sufficient to record the fact that organs other than these two may respond to the same stimulus, shock or other, in a manner resulting in ischemic necrosis.

SUMMARY

Three experiments were carried out to investigate a possible relationship between the Shwartzman phenomenon and purpuric meningococcemia.

Experiment 1 served to confirm and elaborate the fact that twice-washed meningococci, both living and dead, possess potent preparatory and provocatory substances capable of producing the local Shwartzman phenomenon.

Experiment 2, by comparing the preparatory potency of 18 meningococcic strains, demonstrated that most of the strains (5 of 8) asso-

ciated with purpuric meningococcemia fall into a unique and very potent group. The strains obtained in cases of nonpurpuric meningitis produced less of the preparatory factor.

In serologic group distribution the two categories of meningococci were essentially identical.

Bilateral necrosis of the adrenal glands with hemorrhage was found in 2 animals of experiment 2.

Experiment 3 was designed to test the response of rabbits to meningococcemia maintained, if necessary, over a period of twenty-four hours. General cutaneous purpura was produced in a number of animals. In addition to the cutaneous lesions, 1 rabbit displayed marked adrenal necrosis and hemorrhage: Waterhouse-Friderichsen syndrome.

The close relationship of the general purpura to the local Shwartzman reaction was illustrated by the simultaneous appearance of both in rabbits which previous to their meningococcemia had been prepared in one or two sites by intradermal inoculation of meningococci.

Many of the animals of experiment 3 disclosed at autopsy bilateral renal cortical necrosis. Since in rabbits this lesion is recognized as characteristic of the generalized Shwartzman reaction, it is evident that washed meningococci are capable of producing not only the local but also the general phenomenon.

It is believed that the Shwartzman substance acts directly or indirectly on the interlobular arteries of the kidneys, causing marked vasoconstriction and thus initiating the sequence of events leading to bilateral renal cortical necrosis.

CONCLUSIONS

Washed meningococci, living or dead, are capable of producing the local Shwartzman phenomenon.

The organisms contain more of the potent substances than their supernatant fluids.

Meningococcic purpura has been produced in rabbits. The lesions are interpreted as a cutaneous manifestation of a generalized Shwartzman phenomenon.

The syndrome of Waterhouse and Friderichsen, which may complicate meningococcemia, has been reproduced in a rabbit. The adrenal lesions are considered to be a by-product of a general toxemia and not necessarily a resultant of the Shwartzman mechanism.

It is believed that the appearance of bilateral renal cortical necrosis in experimental meningococcemia is evidence of the ability of the washed bacteria to produce the general Shwartzman reaction. It is furthermore suggested that this lesion is effected not by thrombosis but by vasospasm with thrombosis as a sequel.

MARBLE BONE DISEASE

A Study of Osteogenesis

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PHILADELPHIA

THIS is not intended to be a presentation of a case of marble bone disease. It is an attempt to study a comparatively rare congenital bone disease from the moment of its onset in intrauterine life and through its further stages. It was felt that an ontologic study of congenital diseases may lead farther than the purely morphologic aspect and prove valuable for finding new therapies.

Marble bone disease is also commonly known as Albers-Schönberg disease, as osteopetrosis and as osteosclerosis fragilis generalisata. For the sake of simplification, however, the term "marble bone disease" will be used here. The chief histologic characteristics are: an extremely thick cortex of the long and short bones and a narrow marrow cavity filled to a great extent with medullary bone of abnormal structure. Some of the finer histologic details have been mentioned by various observers; others have up to now escaped attention. Roentgenograms show marked density of all bones, transverse and longitudinal bands of greater and lesser density, and an expansion of the ends of the long bones peculiar to this disease, the so-called clubbing.

The bibliography of marble bone disease up to 1934 has been collected in the excellent survey of McCune and Bradley.¹ Therefore the authors writing before 1934 will not be referred to in this paper except for special reasons. The same restriction will be observed in regard to the authors writing between 1934 and 1946 who did not furnish microscopic descriptions or whose roentgen reports do not contain any new detail.

The case which served for the present study was published by Windholz² during the lifetime of the infant, which was then 11 months old. The roentgenograms showed the typical marble bone changes in all the bones. The clubbing of the ends of the long bones was markedly developed for that age. There was severe atrophy of both optic nerves, but only slight anemia. No data could be obtained on the metabolism

From the Laboratories of the Jewish Hospital.

Part of the expenses of this research was defrayed by the Institut du Cancer, of Paris, France, and by the Jewish Hospital, of Philadelphia.

1. McCune, D. J., and Bradley, C.: *Am. J. Dis. Child.* **48**:949, 1934.

2. Windholz, F.: *Ztschr. f. Kinderh.* **51**:708, 1931.

of calcium and phosphorus. The father and the mother of the child were first cousins. The case must be classified among the malignant types (McPeak³) despite the fact that no fractures had been recorded in the still young infant; it belongs also to group I of Harnapp's⁴ classification, i. e., malignant with probably recessive heredity.

The infant died about two months after it was examined by Windholz, and bone specimens became available for study. A series of cross sections were taken from a femur and a series of longitudinal sections from a rib. The sections were stained with Delafield's hematoxylin and eosin or with Heidenhain's azocarmine modification of the Mallory aniline blue connective tissue stain or were treated with tannin-silver after del Río Hortega's second method.

The cross sections through the middle part of the femoral diaphysis reveal the entire individual history of this bone. In normal development of bone four periods can be distinguished, starting with the first deposition of bone tissue in embryonic life. Each period is characterized by various types of bone tissue, which form an ascending line of differentiation. The timing of the periods naturally varies according to the time at which the various bones begin their ossification. In the femur (Zawisch⁵) ossification (not to be confused with the appearance of the calcification center of the cartilage on the forty-second day) starts in the ninth week with the appearance of a thin periosteal bone sheath or collar around the middle of the cartilaginous model (fig. 1*A*). This is the narrowest part of the cartilage because here calcification started and prevented further expansion. From here calcification and also ossification spread toward the ends. During the first period, vessels from the periosteum grow into the cartilage and remove it by progressive resorption, while from the periosteum new layers of bone are deposited and form the first period cortical stratum. At the end of the first period (fourteen weeks, fig. 1*B*), the cortex consists of various layers of early embryonic plexiform bone. It is thickest in the middle, where ossification began, and tapers out toward the ends. The primary marrow cavity is now formed. It is called primary because it results merely from the resorption of the cartilaginous model and has not yet been widened by resorption of the cortical tissue from within. Moreover, no medullary bone has been formed as yet. (The dark patches in figure 1*B* are blood-filled sinuses.) From now on—i. e., beginning with the second period—the inner layers of the cortex are going to be resorbed from within in order to widen and to shape the (secondary) marrow cavity, while on the periosteal side the cortical strata of the subsequent periods are being deposited. Consequently, there must come a moment when the entire first period stratum as shown in figure 1*B* has been removed and the marrow cavity is bordered by the second period stratum. This point is reached at about five and a half months of intrauterine life. In the end, all the embryonic strata are removed, and the marrow cavity is bordered by fourth period, i. e., lamellar bone. Medullary bone, or spongiosa, starts forming at the beginning of the second period. As all the processes which form and model the secondary

3. McPeak, C. N.: *Am. J. Roentgenol.* **36**:816, 1936.

4. Harnapp, G. O.: *Monatschr. f. Kinderh.* **69**:1, 1937.

5. Zawisch, C.: *Ztschr. f. mikr.-anat. Forsch.* **17**:41, 1929.

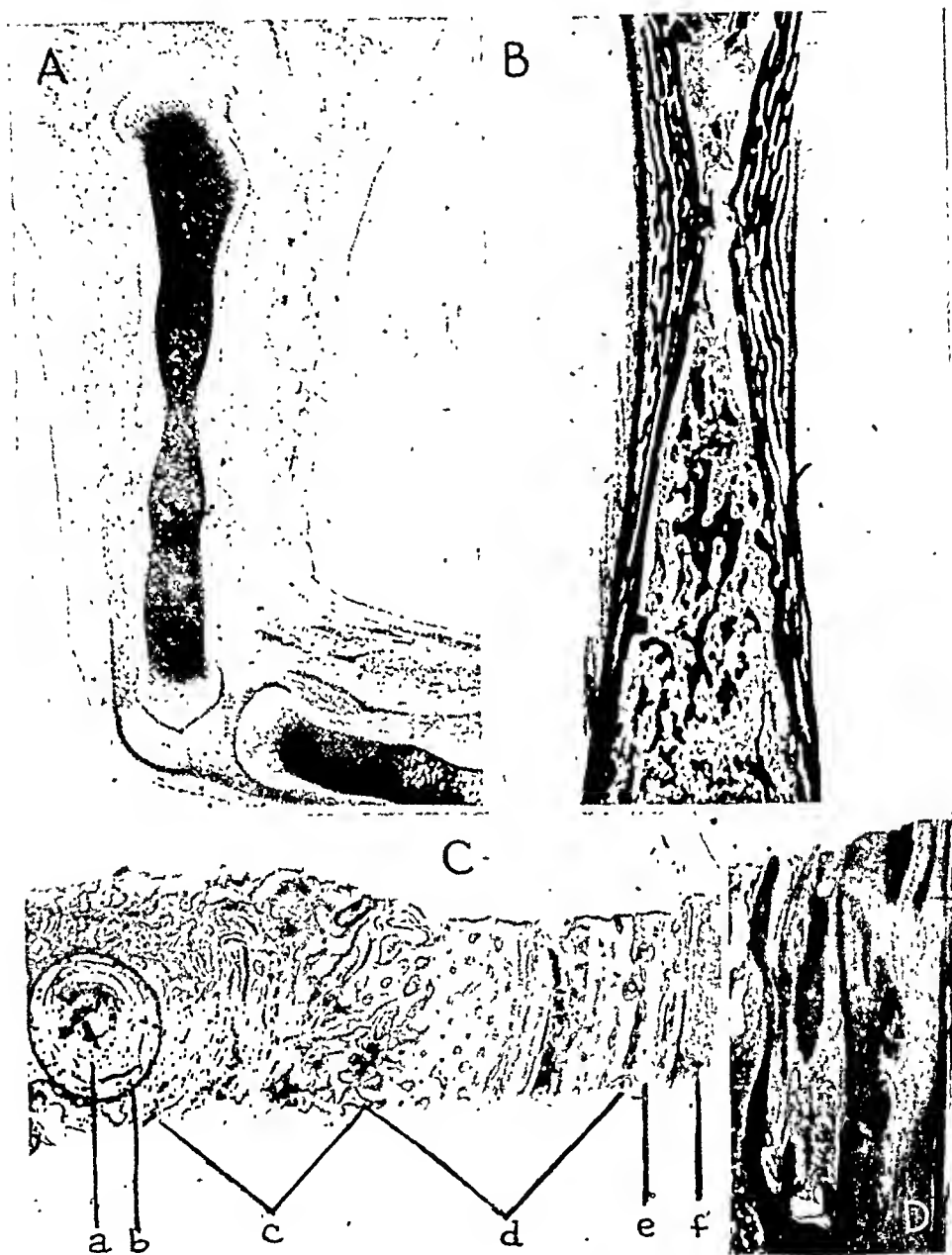


Fig. 1.—A, femur of an embryo at 8 to 9 weeks. $\times 12.5$

B, femur of an embryo at 14 weeks. $\times 12.5$.

C, sector of a cross section through a marble bone femur (middle of the shaft): (a) marrow cavity with trabeculae of the spongiosa and endosteal layers; (b) cement line marking the end of the first period; (c) second period stratum; (d) third period stratum; (e) periosteal stratum deposited during the fourth period; (f) periosteum. $\times 16$.

D, longitudinal section of the diaphysis of a femur of an 11 month old normal infant for comparison with C. To the left are the marrow cavity and remnants of the otherwise resorbed third period stratum. To the right is the actual cortex, consisting of the fourth period stratum. $\times 32$, reduced to $\times 16$.

marrow cavity and the diaphysial cortex start at the middle of the diaphysis, cross sections through this region are the most revealing.

The cross section through the marble bone femur (fig. 1 *C*) shows what happened instead of the normal course of development. The diameter of the marrow cavity corresponds exactly to that of a fetus of 5½ months. It is bordered by a remarkably thick and deeply staining cement line, along which a few layers of endosteal bone have been deposited. This means that the first period stratum was removed normally but that afterward the widening of the marrow cavity was completely arrested. Resorption stopped at the unusually thick cement line between the first and the second period stratum.

The marrow cavity, moreover, contains a few spicules of spongiosa, whereas normally at this place there are none. Both these and the endosteal layers contain basophilic inclusions; i. e., they belong to a less differentiated type of bone tissue, not compatible with the actual age.

The present cortex consists of all the strata of the subsequent periods. This accounts for the unusual thickness of the cortex of marble bones. The strata are not equally arranged around the circumference; they are broader in some areas, narrower in others. The fourth period stratum (*c* in fig. 1 *C*) is particularly thin. It corresponds in size to that of an infant of 5 months instead of that of an infant of 13 months. This seems to indicate that the thickness of the cortex is due merely to lack of resorption and that growth and differentiation were retarded.

For comparison, figure 1 *D* shows a section through the middle of a normal femoral diaphysis of about the same age and at the same magnification. The cortex consists merely of a thick fourth period stratum (pure lamellar bone). All the preceding strata have been removed, and only a few remnants protrude like spicules into the narrow cavity.

The strata in marble bones do not merely persist; they are also pathologic in their structure. The second period stratum (fig. 2 *A*), normally consisting of late embryonic plexiform bone, is mixed here to a great extent with chondroid bone. Chondroid bone of this type represents the lowest degree of differentiation in bone development. Normally it appears in the second period at the metaphysial ends of the cortex, where the bone grows in length. It plays a merely transitory role and is soon superseded by the ongrowing diaphysial cortex. Here it has been formed throughout the diaphysis and during all of the second period. Besides being out of place, it is also malformed. The most striking feature is the intense basophilia of its ground substance; i. e., the pH is lowered.

The stratum which corresponds to the third period (fig. 2 *A* and *B*) is less severely malformed and in places appears even grossly normal. It consists of late embryonic plexiform bone, the interspaces of which are filled, or in the process of being filled, with prelamellar and lamellar bone belonging to the fourth period. However, patches of chondroid bone reappear here and there. This means that the bone-forming blastema had begun to recover in the direction of normal differentiation but that relapses occurred.

The stratum deposited during the fourth period (fig. 2 *B*) is normally differentiated and consists of lamellar bone, but it is poorly developed. In the part of the periphery shown in figure 2 *B* it is even thinner than that shown in figure 1 *C*, and in other parts it is missing altogether. The reversion to normal differentiation, although belated, is also shown by the fact that haversian systems and even rudiments of a mosaic structure have begun to form in the vascular spaces of all the strata, even within the chondroid bone of the second period stratum.

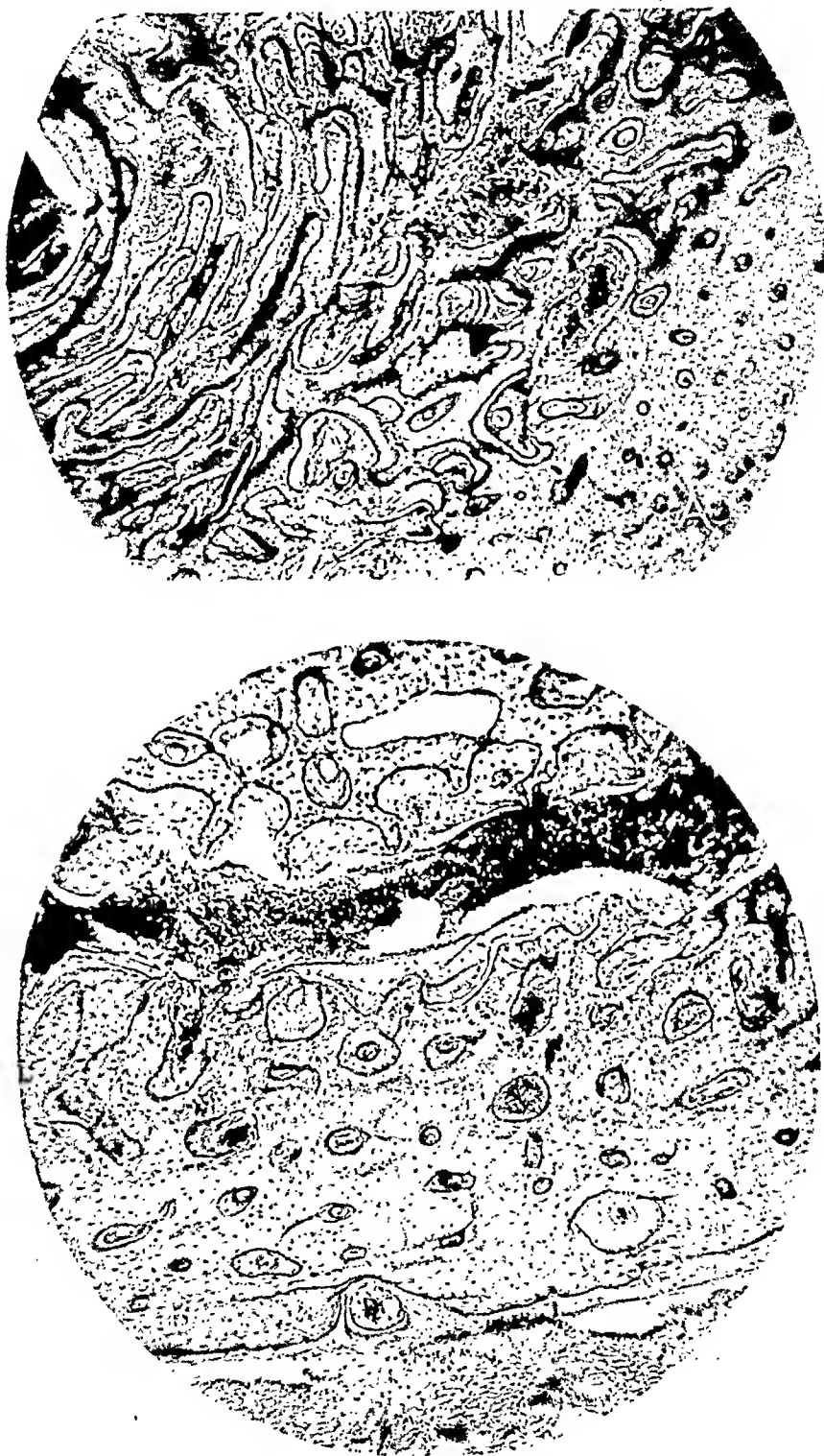


Fig. 2.—*A*, marble bone femur. It shows the second period stratum with adjacent parts of the marrow cavity (left) and the inner layers of the third period stratum. $\times 50$.

B, marble bone femur showing the third period stratum with a large resorption space between the inner and the outer layer; also seen is a fourth period stratum with haversian lamellas. $\times 75$.

In all the strata the unusually thick and deeply staining cement and junction lines are conspicuous. This phenomenon is likewise more pronounced in the inner strata and diminishes toward the periphery.

The various types of bone tissue forming the strata are pathologic in their finer histologic structure. Only the most striking of these details can be mentioned.

The osteoblasts are everywhere so flat that they are hardly distinguishable. In fact, they have escaped the attention of a number of observers, who have affirmed that there are none in marble bone disease. In this connection it must be mentioned that flat osteoblasts as such are not necessarily pathologic. Normally, the size and the shape of the osteoblasts depend on (*a*) the degree of the differentiation of tissue, and (*b*) the state of function (Zawisch⁶). The osteoblasts of the less differentiated types—chondroid and transition bone—are flat or spindle shaped originally, and the flattest osteoblasts are those which produce cement and junction lines. Cuboid osteoblasts are typical merely for the higher differentiated forms at the peak of function. They also become flat when exhausted. Therefore, flat osteoblasts may be considered as pathologic only if they are present in more highly differentiated types of bone tissue and throughout sizable areas. Otherwise they may indicate merely a regional ablastic period such as those which normally occur between productive periods. In the present case no other than flat osteoblasts could be found anywhere; therefore, they are certainly pathologic. There are also fewer than normal.

The osteocytes in chondroid bone are crowded, but in all other types of bone they are somewhat less numerous than normal. They also more often melt into ground substance. This can be seen comparatively easily in sections treated with tannin-silver. The same method shows that the canaliculi are longer and thinner than usual and that they form a denser network of anastomoses. This, according to Veit,⁶ shows that the nutrition of the tissue is somewhat impaired. This is due to the relative paucity of osteocytes and the distance of central parts of the bone tissue from vessels after long periods of deposition. Therefore the network of canaliculi is stretched in order to make up for the deficiency.

In lamellar bone the individual lamellas are thinner than normal and densely packed. This is one of the reasons for the "density" of marble bone and explains why it is so hard to cut despite good decalcification.

Polarized light⁷ reveals a relative scarcity of fibrils in all the bone tissues. Chondroid bone of the type which is hyperplastic in marble bone does not contain fibrils even normally, and the pathologic change in the ground substance consists here merely in an increase of basophilia. In the other bone tissues the scarcity of fibrils is very conspicuous. It diminishes with the recovery of the blastema, i. e., better differentiation, but is still marked even in lamellar bone. This scarcity of fibrils has a bearing on the calcium content of marble bones, as will be seen later. It is probably related to another phenomenon. There are indications that the substratum which furnishes the preosseous substance is less viscous than normal and precipitates more readily. But this leads into the domain of leptology, where, as regards bone substance, little is known.

It may be mentioned, because it is sometimes contested in the literature, that osteoclasts are present in all stages of their evolution; budding, active and exhausted.

6. Veit, O.: Inaug. Dissert., Freiburg i. Br., University Library, 1934.

7. Prof. G. B. Wislocki, head of the department of anatomy of Harvard Medical School, permitted me to use the polarization microscope of the department.

These finer details seem to indicate at least one thing: that all the various steps of the deposition process are taken at a stride and that what may be called the microrhythm is accelerated. A more detailed explanation of these phenomena cannot be given here.

The malformation of the bone is in itself responsible for the general retardation of resorption in marble bone disease. Resorption and deposition are two phases of one and the same bone-building process; they condition and stimulate each other by the biochemical changes which each phase produces (Zawisch⁸). If one of the phases is disturbed, the other will suffer likewise, and the entire process, in a vicious circle, becomes disrupted. This is evidently the case in marble bone disease. In normal bone, resorption occurs at a certain level of stimulation. In marble bone disease this threshold of response to the stimuli which bring about resorption is raised. In this femur the resorption of bone adjacent to the marrow cavity is completely arrested by the cement line deposited at the end of the first period. Internal resorption such as is normally found at the base of the internal reconstruction process is retarded. This retardation is due partly to the thickness of the cement and junction lines, which even normally act as a kind of temporary barrier against resorption, and partly to the biochemical properties of the pathologic bone tissues. Nevertheless, it follows even here certain general rules, one of which is that the longer a period of deposition lasts, the longer and the more active is the following period of resorption. Therefore, abnormally large resorption spaces are formed locally from time to time within the strata. (Compare *A* and *B* in figure 2.) Several such periods of resorption can be distinguished. The latest is the one that occurred in the second period stratum, where large, fresh and highly irregular resorption spaces can be seen. This stratum, the most pathologic, opposed the stiffest resistance to resorption. In the third period stratum, in a part of the periphery, not showing in figure 2, a phase of most energetic resorption occurred and came to a standstill some time ago. Large former absorption spaces are filled now with young, to a great extent lamellar, bone. These spaces, all of the same age, were confluent and irregular, so that during this period the stratum must have presented the aspect of osteoporosis. Indeed, temporary osteoporosis occurring in marble bone disease has been reported by several authors. Moreover, there is a large space between the outer and inner layers of the third period stratum (fig. 2*B*), filled with hemopoietic marrow. This space is present throughout the entire circumference at the level of the series of cross sections, and it is in open communication with other spaces containing blood-forming elements, whose joined surface area exceeds by far that of the marrow cavity at the level of the sections, so that they can be considered as an accessory marrow cavity.

8. Zawisch-Ossenitz, C.: Wien. klin. Wchnschr. 47:801, 1934.

In the scanty fourth period stratum resorption appears normal, thus emphasizing once more the tendency to recovery.

Thus, the findings in the femur seem to indicate that marble bone disease starts at the beginning of the second period of bone formation. For the femur, this time is the middle of the fourth month of intrauterine life. By that time the thick cement layer had been laid down on the first period stratum, and from then on pathologic bone tissue was deposited. The first period stratum was probably normal, because it was normally resorbed. After this, further differentiation and growth were retarded. Resorption of the stratum from within did not take

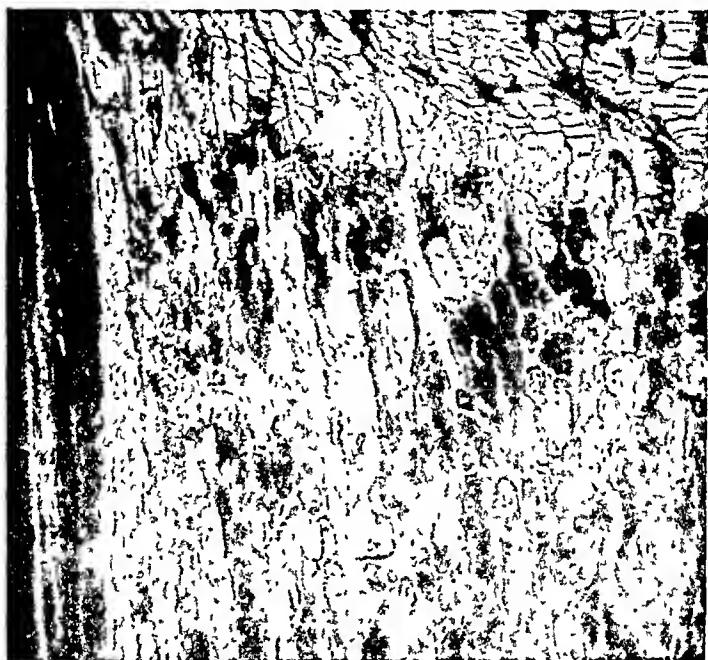


Fig. 3.—Marble bone rib, part of a longitudinal section. Note the epiphysial line and the densely packed spongiosa. To the left, cortex and periosteum. Azan-Mallory stain; $\times 60$.

place; therefore the cortex remained unusually thick. Internal resorption cannot make up for this failure and the entire bone-forming process remains disrupted.

The metaphysis always remains behind the diaphysis in development and differentiation. Therefore it can be expected to show the earlier phases of the development of marble bone still in existence and to furnish the clues for the development of the hyperplastic spongiosa. Figure 3 is a longitudinal section through the rib, taken from the chondroid-costal end. In the rib, ossification starts later in embryonic life than in the femur; therefore, the various stages are here even younger than in the femoral metaphysis.

The thin cortex of this part of the rib consists almost exclusively of second period chondroid bone of the same pathologic structure as that found in the femoral diaphysis. At the age of thirteen months it should be composed of

lamellar bone with remnants of chondroid and transition bone at the metaphysial edge around the cartilage. Therefore, the metaphysial cortex is still in its second period, the chondroid bone has never been resorbed, in order to make place for more highly differentiated tissues. Only slight attempts at further differentiation can be seen on the side of the periosteum.

The absorption front of the cartilage is normal, but the succeeding endochondral ossification is pathologic from the beginning. Normally the first period stage, in which no endochondral bone is formed, is being repeated here as long as the bone grows. The capillaries which absorb the cartilage always keep ahead of the osteoblasts, and deposition of bone begins at a certain distance from the absorption front. Thus the capillaries have time to absorb also a certain amount of cartilaginous ground substance. Here, as soon as a cartilage cell is liberated by absorption of its walls, basophilic matter and an osseous globule are deposited in the cavity (fig. 3, azocarmine method). Thus the cartilaginous ground substance is immediately sealed off against further absorption. Lying close to each other because no larger spaces have been created, the osseous globules coalesce almost immediately, and further deposition contributes to this process. The result is an extremely dense network of medullary bone which fills the entire marrow cavity.

This deposition of basophilic matter which in endochondral ossification reinforces the slender remnants of cartilaginous ground substance before real bone tissue is being deposited is also a characteristic of the second period formations. It is not of itself a pathologic formation, as some observers believe. It is the hyperplasia which is pathologic. The equivalent in the cortex is transition bone; therefore some observers mistake cortex for spongiosa.

Endosteal ossification in marble bone disease, therefore, shows the same pathologic development as cortical. A hyperplastic production of less differentiated bone tissue, containing more basophilic matter than usual, is at the root of the evil. That it also chokes off resorption is evident in this rib. Normally, waves of resorption, starting in the regions nearest the center of the bone and proceeding toward the ends, remove all superfluous spongiosa. They leave only such trabeculae as, after further differentiation, are being shaped into the typical trajectorial structures at the end of the shaft. Here no such resorption has taken place, and the marrow cavity is densely packed with spongiosa as far as the sections reach. The fact that even in the center of the shaft of the femur some spicules of medullary bone were found fits into this picture. Resorption of spongiosa, inhibited for a long time, never catches up completely.

A further tissue differentiation, corresponding to the age of the child, could not be found. In summary it can be said that the metaphysis of the rib shows the earlier stages of the process which could be retraced in the diaphysis of the femur and which accounts for the hyperplasia of medullary bone.

COMMENT

The literature in general seems to bear out the thesis advanced here that marble bone results from a specific second period disease of the

bone-forming blastema. Unfortunately, the spongiosa is more often described than the cortex, and no detailed description of the developing cortical bone can be found. A number of authors have maintained that marble bone disease is a malformation of "endochondral" bone only, meaning the spongiosa, and some writers have mistaken cortex for spongiosa because of the hyperplastic production of less differentiated bone tissues present in both. Others have expressed the belief that even these tissues—chondroid, osseous globules and basophilic inclusions—are pathologic of themselves. Therefore they are sometimes given special names which are hard to transcribe, whereas the proper terms exist and the pathologic changes consist in the hyperplasia and the malformation of these tissues. All this at times confuses the issue.

However, in the cases which have been reported since 1934 and in which microscopic observations have been described, the age ranging from birth to senescence, the findings corroborate the description given here and show fairly well the further evolution of marble bone disease.

The most thoroughly analyzed case is that observed by Dijkstra.⁹ The patient was a child 3 years old. A rib, a vertebra and an unspecified piece of a cranial bone were examined. In the rib the state of the endosteal ossification was the same as in the Windholz case described here. But the cortical ossification was more advanced, the stratum consisting of late embryonic plexiform bone with a little lamellar bone. Dijkstra described it as *semblable à celui du fœtus normal* (similar to that of a normal fetus), and indeed it corresponded to the third period, whereas in the normal rib of this age lamellar bone prevails. Differentiation had taken place but had not yet caught up.

The vertebra of Dijkstra's case is of considerable interest. It presented the characteristic double cone form of the primary marrow cavity which Dijkstra was first to describe for marble bone disease. The vertebrae begin ossification later than the long bones of the limbs. Dijkstra's specimen corresponded approximately to the femur presented here, only the double cone marrow cavity, pulled out by the great elongation of the femur, was clearly visible in the short vertebra. It was completely filled with spongiosa. The cortical stratum was formed by late embryonic plexiform bone with little lamellar bone. Thus differentiation was a little behind that of the rib, which corresponded to the ossification rates of these bones. Dijkstra's description of the marrow cavity induced Schmidt¹⁰ two years later to suggest that the double cone form is at the basis of the well known clubbing of the ends

9. Dijkstra, O. H.: *Ann. d'anat. path.* **12**:131, 1935.

10. Schmidt, M. B., in Henke, O., and Lubarsch, O.: *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1937, vol. 9, pt. 3.

of long bones. However, other explanations for the clubbing are better substantiated, as will be shown.

Dijkstra showed an interesting feature in the bone of the calvaria. He distinguished three main periods of resorption in the normal development of the flat bones of the skull. The first concerns the inner table and models the curvature. In the second the plexiform bone is resorbed and replaced by lamellar. In the third the normal shape of the diploe is achieved by development of lacunas. In the case which he studied, the first period had developed normally, but the second and the third were greatly retarded and no diploe in the real sense was present. In the stratum corresponding to it, lamellar bone had already developed. In the cranial bones ossification starts earlier than in the vertebrae. The state of the marble bone changes in the calvaria corresponded to this situation. And, although a detailed analysis cannot be made here, the three stages of ossification in marble bone disease—normal beginning, malformation, repair—can be clearly distinguished in Dijkstra's description.

In Gerstel's¹¹ patient, a child $3\frac{1}{2}$ years old, basophilic formations, probably even chondroid particles, were present even in the calvaria. This is not surprising, since they appear here also in normal ossification at very early stages. But they disappear soon. Their presence in a marble bone calvaria of this age shows that the process is particularly severe with respect to the bone changes. There are certainly various degrees of the bone malformation, and these degrees do not of themselves affect the fitness of the subject for survival. The rest of Gerstel's observations do not offer new details but provide a complicated nomenclature which is not possible to translate.

Clifton, Frank and Freeman¹² observed their patient almost from birth, and the child was $5\frac{1}{2}$ years old when parts of the tibial metaphysis and of a rib near the costochondral junction were taken for biopsy. According to the size and the localization of the specimens, information is scanty. The cortex of the rib is still formed by chondroid bone; so differentiation in this case was particularly belated. The authors observed the basophilic inclusions and their character as neoformations with great accuracy. They stated, without arguing on the subject, that at some distance from the absorption front these formations were thicker than the slender cartilaginous remnants in the immediate vicinity. As such distinctions are not always easy to make in normal bone, it is pretty evident that the authors have seen the hyperplasia. In the tibial metaphysis the cortical tissue consists of late embryonic plexiform bone with chondroid inclusions. As differentiation of the tibial metaphysis normally begins later than that of the central parts of the bone and, on the

11. Gerstel, G.: *Frankfurt. Ztschr. f. Path.* 51:23, 1937.

12. Clifton, W. M.; Frank, A. A., and Freeman, S.: *Am. J. Dis. Child.* 56:1020, 1938.

other hand, is in advance of that of the metaplysis of the rib, this particular state of differentiation is just what one would expect in the marble bone tibia of a young child. But the absence of any differentiation in the rib suggests that the process was severer in this case than in the Windholz case with respect to the bone changes, and yet the child was alive at the age of 5½. Of the resorption process nothing is revealed besides the fact that no continuous marrow cavity is present.

The case of Lamb and Jackson¹³ does not add any new facts. In the picture of the tibia of the 3½ year old child, the basophilic inclusions in the numerous and densely packed spongiosa trabecula are so large that their character as hyperplastic neoformations and the absence of any rebuilding process can be seen at the first glance.

The vertebra of van Creveld and Heybroek's¹⁴ first patient, 9 days old, shows the same gross structure as that of Dijkstra's⁹ patient; the authors only describe it the opposite way. Two wedges of bone (cortical) with the points directed toward each other "compress" the endochondral trabecula of the center. They expressed the belief that this cortex was normal but stated that the "cartilaginous rests" in the spongiosa were thicker in the center of the vertebra than at the periphery.

A small number of authors had an opportunity to study marble bones of adults. Laubmann¹⁵ observed the disease in a young man of 25. He is one of those who, not having studied fetal bones, describe normal features as pathologic per se and apply to them a vague terminology which makes an analysis difficult. Essentially, Laubmann's case presents the same microscopic characteristics as those described by his predecessors. A picture of his vertebra shows rests of chondroid bone in the otherwise evolved cortex, which is remarkable in one of that age.

Heidger's¹⁶ case was that of a man 58 years old. It is hard not to rule it out altogether as a case of marble bone disease. Certain details which the author described reminded him of rickets, and broad fresh deposits of primary bone substance (unfortunately called "osteoid"¹⁷ by him) suggested to him that the aged man had also suffered from osteomalacia. Actually the patient died of bone abscesses and multiple myeloma. What remains of the marble bone features were remnants of what the author called "calcified cartilage" despite the fact that they were obviously larger than any real rests of cartilage ground substance

13. Lamb, F. H., and Jackson, R. L.: *Am. J. Clin. Path.* **8**:255, 1938.

14. van Creveld, S., and Heybroek, N. I.: *Acta pædiat.* **27**:462, 1940.

15. Laubmann, W.: *Virchows Arch. f. path. Anat.* **296**:343, 1935.

16. Heidger, P.: *Beitr. z. path. Anat. u. z. allg. Path.* **97**:509, 1936.

17. The tragic fate of the word "osteoid" becomes more and more obvious every year. Originally meant by Koelliker, in the 1880's, to designate the cell-less bone of fish, it can be found in recent papers on bone disease for almost anything besides lamellar bone.

can be. But the author affirmed that they were "metaplastic neoformations of cartilage" because they were never found in desmal bones. This is confusing, for they are neoformation, but not of cartilage, and they do appear also in desmal bones in normal osteogenesis as well as in marble bone disease.

Heidger mentioned deeply staining cement and junction lines; Schmidt¹⁰ described them also, as particularly thick. Heidger's case showed "eburnation," thus confirming preceding descriptions of the adult marble bone cortex and its "mosaic structure," i. e., a composition of relatively small and numerous haversian systems (osteons of modern terminology). Schmidt mentioned that the vessels are more numerous and more ramified than normal. That is precisely what causes the mosaic structure, because each osteon is formed around a vessel.

Thus the information on adult marble bones since 1934 is rather poor. But, pieced together with the information gathered before that time, it conveys a fairly good general impression of the process of repair. McCune and Bradley¹ enumerated the known patients whose cases have been reported, with respect to their ages, from the newborn infant up to the adult. It can be observed that in a general way the more advanced the age of the patient the more often the words "lamellar bone" and "haversian systems" occur in the description. Thus differentiation proceeds if the patient survives, but the mosaic structure reveals that during the period of interior reconstruction resorption and deposition continue in a pathologic rhythm and on a pathologic scale. The thickness of the cortex shows that resorption lags behind for a long time. Remnants of less differentiated bone tissues may or may not be present in the adult bones. In a single case, that of the 25 year old patient of Clairmont and Schintz (see McCune and Bradley¹), the microscopic structure of the bone was described as "completely normal save for the increased branching of the haversian canals." But the material in this case consisted of a mere fragment obtained for biopsy from a not specified place in the femur.

In how far the finer details of the histologic structure mentioned on page 60 (fibrils, osteoblasts, osteocytes) also become more normal in the course of repair is hard to tell. They have not been investigated before, and I had no opportunity to study adult marble bones.

There is as yet no uniformity in the opinions of the various commentators as to whether marble bone disease is based primarily on a faulty bone-building process or on a primary lack of resorption. More precisely: Is it the deposition phase or the resorption phase which is at fault and, consequently, is it the bone-forming blastema or the vascular system which is diseased?

Most authors have confined their concepts to endochondral and endosteal ossification. McCune and Bradley¹ stated that the disturbance responsible for the sclerosis seems to be located in the endosteal and endochondral metabolism of bone: "The most widely accepted current concept holds that the fundamental defect in Albers-Schönberg's disease consists in a faulty differentiation and development of this primitive substance," i. e., the mesenchyma. Full credit for this clear formulation is due to the authors, because none of their predecessors had expressed it directly in this way.

In 1937 Schmidt¹⁰ summarized his opinion and that of most authors by stating that Albers-Schönberg disease is the result of a lack of resorption. Nussey¹⁸ one year later said that most authors have ascribed the departure from normal to a disturbance of endochondral formation. Dijkstra⁹ decided for delayed resorption as the primary cause, and so have in recent years van Creveld and Heybroek.¹¹ Of the other writers mentioned here, most have been in favor of a primary disturbance of endochondral formation; only Gerstel¹² explained the disease by coining yet another word, *Dyssynusis*, which means a disturbance in the equilibrium of the tissues.

The modern concept of the bone-building process as a unit, the two phases of which—deposition and resorption—condition and regulate each other, tends to level out the discrepancies in the explanation of marble bone disease. Wherever deposition is abnormal, resorption is adversely influenced and vice versa. From the analysis presented here, it seems that the vascular system is not primarily affected, because resorption proceeds normally up to the moment when the blastema starts to function in a pathologic way, and then it becomes disrupted. In this sense, therefore, McCune and Bradley's formulation, that the fundamental defect is faulty differentiation and development of the mesenchyma, holds good and is much to the point.

The best observers, when venturing a conjecture, place the onset of the disease at an early stage of intrauterine development. It has been shown here that all the available data point to the second period of bone development as the time when the faulty differentiation begins. The question is whether the noxious influence acts during a certain period of intrauterine life and hits the bones which during that period are in a state of response, or whether it invariably attacks all the bones in the second period, regardless of the time at which the respective second period of each begins. In the first case, possible clues to the etiologic factor and the therapy might be found in the condition, hormonal or otherwise, of the mother. In the second case one is faced by the general problems of the "vitia primae formationis," of which in the special case

18. Nussey, A. M.: Arch. Dis. Childhood **13**:161, 1938.

of bone formation one does not know the first word. One is ignorant of the embryologic factors of induction and competence responsible for this process.

Unfortunately, investigators will probably have to decide for the second possibility. It is true that the clavicle, the bone first to begin ossification (at the start of the sixth week), has never been examined microscopically in cases of marble bone disease, and neither have the carpals, which begin latest (after birth), nor the epiphyses of the long bones. But the available roentgenograms show the clavicles to be diseased in the same way as the other bones, and they suggest this also for the epiphyses. This constitutes a wide range of time for the development of the disease. Therefore, one will have to conclude that it is a general failure of the fetal bone-forming blastema which becomes manifest whenever a bone enters its second period, i. e., when, at a certain level of differentiation, it is in a state of response to the noxious influence.

But as long as there are no histologic and developmental data concerning the bones and the epiphyses which ossify after birth, the last word in answer to this question remains to be said. It is to be hoped that in the next cases which come to the attention of observers, these bones will be examined likewise.

That the words "heredity" or "hereditary factor" present no real solution is clear. There is no doubt that many patients with marble bone disease show recessive heredity, but others do not. This merely proves that the real etiologic agent arises spontaneously in certain ones and may or may not become a transmissible character; it does not specify what this agent is. The word "heredity" is nothing but a shrug of resignation.

The roentgen picture of marble bone disease can be readily explained on the basis of the present microscopic observations. It comprises: the clubbing, the density of the bones in general and the bands of greater and lesser density within individual bones.

The clubbing of the ends of long and short bones has been explained by Schmidt¹⁰ with reference to Dijkstra's¹¹ vertebra as resulting from the narrowing of the central part of the shaft. This is not quite to the point, because the shaft is not much thinner than normal, whereas the metaphyses are much thicker. This spreading out of the metaphyses gives the typical roentgenographic shadow known as clubbing and if the patient survives the epiphyses follow the broadened outlines.

The metaphysis is the place where normally the less differentiated types of bone are produced which provide for rapid growth. The amount always corresponds to the rate of growth. It is therefore greater in that end of a long bone which—as Ollier¹⁹ had shown—grows more rapidly

19. Ollier, L.: *Traité expérimental et clinique de la régénération des os*, Paris, V. Masson & Fils, 1867.

than the other, e. g., the proximal end of the humerus or the distal end of the femur.

In marble bone disease, bone tissues of these types are hyperplastic. Consequently, as Cortes-Llado²⁰ has shown, the clubbing is more marked in those metaphyses which, according to the rules of Ollier,¹⁹ grow more rapidly. These facts can be verified on all the roentgenograms published after 1934—e. g., in the papers of Shallow, Davis and Farrell,²¹ Pounders,²² Wortis,²³ Clifton, Frank and Freeman,¹² van Creveld and Heybroek¹¹ and especially Cortes-Llado.²⁰

The phenomenon shows also in the case presented here. Figure 4 gives the outlines of the roentgenographic shadows of the femur and the humerus published by Windholz.² The clubbing is more marked

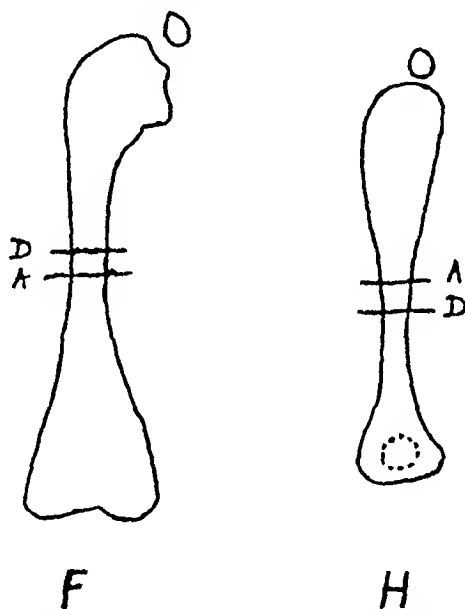


Fig. 4.—Outlines of the roentgenographic shadows of femur (*F*) and humerus (*H*) in marble bone disease: *A*, anatomic median line; *D*, developmental median line.

at the distal end of the femur and at the proximal end of the humerus. Moreover, another detail can be seen here and verified in the roentgenograms of other writers. The anatomic median line *A* divides the entire bone in two equal halves. The developmental median line *D* does so for the shaft alone and therefore marks the place where ossification started in fetal life. The developmental median line has receded toward the less rapidly growing end, i. e., toward the proximal metaphysis of

20. Cortes-Llado, A.: *J. internat. de chir.* 1:63, 1936.

21. Shallow, T. A.; Davis, W. B., and Farrell, J. T., Jr.: *Ann. Surg.* 100:512, 1934.

22. Pounders, C. M.: *Ann. Int. Med.* 8:966, 1935.

23. Wortis, H.: *Am. J. Dis. Child.* 52:1148, 1936.

the femur and the distal metaphysis of the humerus. In other words, the more rapidly growing ends are longer. That this difference in growth ratio is greater in these two halves than in normal bones has been clearly shown by Cortes-Llado²⁰ in a case which he observed during ten years. The author summarizes his observations by stating that the bones attacked by marble bone disease follow the rules of Ollier on a larger scale. They are subject to the same morphogenetic influences as normal ones but present the resulting phenomena in an exaggerated way. The difference in the growth ratio of the two ends is not the same in all bones. In figure 4 it can be seen that the difference between *A* and *D* is greater in the humerus than in the femur. This is also mentioned by Cortes-Llado for his case, and it can be seen in the roentgenograms of several other authors. An explanation of this special detail on the ground of normal development is not available at present.

The relative slenderness of the shaft of the long bones despite the thickness of the cortex is the result of the belated differentiation and its slow progress. This is not incompatible with the statement made previously that the microrhythm of deposition is accelerated, for this productive process spends itself with equal rapidity, whereupon an ablastic period follows.

The second characteristic of the roentgenogram is the "density" of the marble bone. Various components seem to be involved. Where lamellar bone is present, there is the density of the gross structure, i. e., the densely packed osteons (mosaic structure) and the dense microstructure of the individual osteon, i. e., the thin and closely packed lamellas. But this is evidently only part of the explanation. There is the problem of the calcium content of marble bones. That this is higher than normal has always been surmised but never definitely proved. Histochemical methods have not been used up to now, and the general ash contents have not been analyzed except by Clifton, Frank and Freeman,¹² who made use of a biopsy fragment for this purpose. They showed that the spongiosa has a higher ash content than the cortex.

The calcium and phosphorus metabolism test does not give any clues concerning the actual calcium content of the bones in marble bone disease. The results of the test are extremely variable, and the last decade has not brought forth any new evidence in this respect. Therefore, the opinion of McCune and Bradley¹ still holds good, that there is no way to determine, at present, whether it [the disturbance of the calcium and phosphorus metabolism test, if present] "is related directly to the course of the disease or whether it is simply an effect of the exaggerated production of bone." To this must be added that an abnormal outburst of resorption such as may occur in the course of the disease must needs mobilize a larger amount of calcium than usual. Therefore, it can be reasonably assumed that the variability of the results

of the test in cases of marble bone disease is due to the various states of the bones during the period of investigation. The results of the test must depend entirely on which phase of the exaggerated bone-forming process—deposition or resorption—prevails at that time.

The scarcity of fibrils in marble bones, however, reveals that the bones must indeed be richer in calcium than normal bones. The calcium salts are precipitated in the binding substance and not in the fibrils. If, as in the case of marble bones, the relative amount of binding substance is greater, these bones must contain more calcium than usual. Moreover, those types of tissue, e. g., chondroid bone and the basophilic inclusions, which even normally do not contain fibrils, are hyperplastic in marble bones, and this likewise must increase the absolute amount of calcium. In this way the results obtained by Clifton, Frank and Freeman,¹² indicating that the spongiosa has a higher ash content than the cortex, are explained; the spongiosa is richer in basophilic inclusions than the compacta.

The third characteristic of the roentgenogram is given by transverse and longitudinal bands of greater and lesser density. (See McCune and Bradley¹ and after 1934 Shallow, Davis and Farrell,²¹ Root,²¹ Schmidt,¹⁰ Cortes-Llado,²⁰ Wortis,²² Nussey,¹⁸ Clifton, Frank and Freeman,¹² van Creveld and Heybroek¹¹ and others.) The more frequently seen are the transverse bands which run through the metaphysis of long bones parallel to the epiphyseal line. In rarer cases longitudinal lines are found in the diaphysis parallel to the periosteum. Sometimes both types are present in one and the same bone. In flat bones such bands are seen as following the outlines.

It is clear that the transverse bands concern the spongiosa, the longitudinal bands the cortex. No microscopic evidence exists up to now to explain the former. McCune and Bradley¹ expressed the belief that the transverse stratification is the expression of the intensity of (a) longitudinal growth and (b) osteoblastic production and osteoclastic resorption. Cortes-Llado²⁰ said that it is the expression of the osteogenetic activity at the absorption front, which in fact means the same thing.

For the longitudinal bands present in the case discussed here, an explanation can be given. Windholz² called them *periostale Appositionsstreifen* (periosteal deposition bands). The word is badly chosen since it would imply that the depositing of bone under the periosteum is pathologic in itself, which of course it is not. What is meant is the presence of a subperiosteal zone of greater density which is apposed to an inner zone of lesser density. Now this corresponds exactly to the microscopic structure of the femoral cortex as shown in figure 1 C and

24. Root, J. H.: Am. J. Dis. Child. 49:964, 1935.

2 *A* and *B*. The subperiosteal zone of greater density in the roentgenogram corresponds to the fourth period stratum plus the outer layers of the third period stratum. Then follows a zone containing large resorption spaces, as shown in figures 1 *C* and 2 *B*. This is bound to be expressed in the roentgenogram as a band of lesser density. Finally the inner strata must have appeared denser again. The resorption spaces in the innermost second period stratum appear so fresh that they probably were not present as yet when the roentgenogram was taken.

Therefore one can probably enlarge on the explanation of McCune and Bradley¹ and of Cortes-Llado²⁰ and say that the bands of greater and lesser density seen in the roentgenogram are brought about in two ways: (*a*) by periodic remissions and recrudescences of the pathologic deposition of bone and (*b*) by periodic recurrences of widespread resorption.

Such outbursts of resorption have been described microscopically by several authors (Schmidt¹⁰). That even osteoporosis occurs at times in marble bone disease was proved microscopically in the case of Kudrjawtzewa (McCune and Bradley¹). This gives a clue to the apparently paradoxical fragility of marble bones. Fractures must occur more readily during a period of widespread resorption. Moreover, marble bones must be more brittle than normal bones because they are poorer in fibrils. Fractures in marble bones, for the most part, do not splinter, and the bones when pounded break up in the irregular fashion of glass, so that no definite planes of cleavage can be discerned (Clifton, Frank and Freeman¹²). This also points to the lack of fibrils.

It remains to be considered whether and in how far the anemia which generally accompanies marble bone disease can be explained by the developmental picture given here.

The question is linked in a way to that of the other forms of osteosclerosis combined with blood diseases, e. g., leukemia or pseudoleukemia. This part of the problem cannot be examined here. For the latter group of diseases too little histologic evidence is available, so that nothing is known about the relationship between the bone and the blood disease in these conditions. As Jordan and Scott²⁵ have put it, one cannot decide as yet for any one of four possibilities: The association is purely accidental, or osteosclerosis is the result of the blood disease, or the blood disease is the result of osteosclerosis, or the two processes occur at the same time and are due to the same etiologic agent.

Applied to marble bone disease, the two first possibilities can be ruled out. The third, that anemia is the result of the bone disease, has been understood up to now merely in the sense that a lack of space in the marrow cavity prevents the formation of a suitable amount of blood

25. Jordan, H. E., and Scott, J. K.: Arch. Path. 32:895, 1941.

elements. As this explanation would cover merely the quantitative changes in the blood and not the qualitative, McCune and Bradley¹ expressed the current opinion by saying that only one assumption is possible and this is represented by the fourth alternative. Blood and bone have a common forerunner, the undifferentiated mesenchymal cell. The fundamental defect seems to be a faulty differentiation of the mesenchyma responsible for both. McCune and Bradley themselves expressed the belief that the faulty differentiation consists in the production of an excessive amount of abnormal osteogenetic tissue which proliferates at the expense of, or in place of, normal blood-forming elements. It would be, perhaps, simpler to surmise that the same agent which damages the bone-forming blastema at a certain level of differentiation affects equally the blood-forming blastema at a certain level. And this level may be where the hemocytoblast starts to differentiate in the direction of forming red blood cells.

However, modern histology has produced new evidence which is extremely suggestive and points once more in the direction of the third alternative, that the blood disease is the result of the bone disease. In various series of well planned experiments Roehlich²⁶ has shown that the production of marrow is strictly dependent on the resorption of bone. The veins of the cortical tissue open into the sinusoids of the marrow cavity. If this pathway is interrupted by whatever means, or if resorption does not take place, the production of bone-forming elements is arrested. It reappears immediately when resorption begins again and when the venous communications between the interior of the bone and the marrow cavity are reestablished. Roehlich supposed that by the resorption of bone a substance is set free which stimulates the production of marrow when it reaches the cavity by way of the venous channels. Such a substance was postulated by me⁸ when I first described the self-regulating mechanism of bone deposition and resorption. The substance liberated by resorption can very well stimulate not only the formation of blood but also that of bone, and Roehlich's experiments offer corroborating evidence on this point too.

These results have a direct bearing on marble bones, in which defective resorption is most conspicuous. The vicious circle is inaugurated by faulty differentiation and by the production of a pathologic tissue resistant to resorption. The lack of resorption in its turn not only retards further differentiation of bone but also impairs hemopoiesis, because the necessary amount of stimulating substance is not produced. In this connection one may well remember the large resorption spaces filled with hemopoietic marrow within the cortex of the femur shown

26. Roehlich, K.: *Ztschr. f. mikr.-anat. Forsch.* **49**:425 and 616, 1941; **50**: 132, 1941.

here (figs. 1 *C* and 2 *B*). As soon as internal resorption breaks the pathologic barriers and enough bone substance is absorbed, marrow is produced even in places where it normally is not.

This concept would seem to show a way toward a therapy of marble bone disease. If the vicious circle could be broken by stimulating the resorption of bone, considerable improvement of the entire condition could be obtained in those patients who present a chance for survival.

SUMMARY

Marble bone disease is due to the influence of an unknown agent which damages the bone-forming blastema at the beginning of the second period of the development of each individual bone. From this time on, the differentiation of bone tissue is retarded and the less differentiated types become hyperplastic. The malformation is responsible for the general retardation of resorption, because the two phases of the bone-forming process—deposition and resorption—condition and stimulate each other. In marble bone disease the threshold of response to stimuli which bring about resorption is raised. Resorption is arrested at the beginning of the second period for a long time. The results are: fetal size and shape of the marrow cavity, a thick cortex composed of all the embryonic strata which normally are removed, and an unusual amount of spongiosa. The clubbing of the ends of long bones is the result of the hyperplasia and persistence of less differentiated types of bone tissue in the metaphyses. The greater density of marble bones in the roentgenogram is due partly to a greater density of structure when the bones are in a state of repair (eburnation) and partly to a higher calcium content, revealed indirectly by the scarcity of fibrils in all the types of bone tissue involved. The anemia in marble bone disease may be due to the same agent which impairs the differentiation of bone tissue. It is more likely that it is due to the relative lack of a substance which normally is released by the resorption of bone and stimulates the hemopoiesis.

EFFECTS OF ESTRADIOL BENZOATE AND THEIR MODIFICATION BY BLEEDING

Studies on the Skeleton and the Blood Calcium of the Cockerel

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THE EFFECTS of estrogenic substances on bone do not always correspond to the changes in the serum or the plasma calcium level produced by these substances.¹ Therefore, other factors were thought to be involved in the skeletal response to estrogens. These factors may be of chemical or possibly hormonal nature; or local tissue changes, such as decreased vascularization or increased hyalinization of the bone marrow, may play a role in the condensation of the bones occurring under the influence of estrogen.² We thought, therefore, that an induced change in the activity of the marrow, such as hyperplasia after withdrawal of blood, might modify the skeletal effects of estrogens and thus provide some further insight into the mechanism of their action.

The present investigation was undertaken to test this assumption and also to determine whether the skeletal changes caused in cockerels by an estrogen can be correlated with the effects of the estrogen on the plasma calcium.

MATERIALS AND METHODS

Thirty New Hampshire Red cockerels of the same hatch and 30 days old at the beginning of the experiment were used. Each bird was kept in a separate cage and had access to food and water at all times. A starting mash was fed until the birds were 6 weeks old, and a growing mash was furnished from the sixth week until they were killed.³ The birds were weighed at regular intervals.

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1. McLean, F. C., in Luck, J. M.: *Annual Review of Physiology*, Stanford University, Calif., Annual Reviews, Inc., 1943, vol. 5, p. 79. Pindborg, J. J.: *Acta path. et microbiol. Scandinav.* 22:290, 1945.

2. (a) Silberberg, M., and Silberberg, R.: *Arch. Path.* 28:340, 1939; (b) 32:512, 1943. (c) Day, H. S., and Follis, H., Jr.: *Endocrinology* 28:83, 1941. (d) Landauer, W., and Zondek, B.: *Am. J. Path.* 20:179, 1944.

The cockerels were divided into four groups: (1) untreated controls (5 birds); (2) cockerels bled at intervals during the experimental period but not given further treatment (6 birds); (3) cockerels which at the end of a thirty day period of observations received injections of 0.12 mg. of estradiol benzoate^{3a} daily for seven, nineteen or twenty-eight days, the injection being made into the pectoral muscle (8 birds); (4) cockerels bled as were those of group 2 and treated with estradiol benzoate as were those of group 3 (11 birds). Further details of the experimental arrangement are given in the table.

Blood was withdrawn by cardiac puncture as frequently as possible without producing severe anemia. For this reason the intervals between bleedings had to be increased in those birds which were bled for longer periods, in order to avoid the production of serious anemia. The hemoglobin was determined every two or three days by the Evelyn oxyhemoglobin method.⁴

The first determinations of plasma calcium were made at the end of the initial thirty day period of observation and before the first injections of the estrogen were given. Another series of blood calcium values was obtained immediately before the birds were killed. Each value given represents the mean of two or three determinations from samples of the same blood. A fraction of a drop of heparin was added to the blood to prevent clotting; otherwise sufficient amounts of serum could not have been obtained for analysis. The presence of such small amounts of heparin did not interfere with the accuracy of the calcium determination. Calcium was determined by a titrimetric method,⁵ in which perchlorato ceric acid (the solution resulting from the dissolving of a cerium salt in perchloric acid) is used as the oxidizing agent of oxalic acid liberated from a calcium oxalate precipitate. Nitro-ortho-phenanthroline ferrous sulfate complex was used as the indicator. All determinations were made in plasma after ashing with a perchloric acid-nitric acid mixture. Test analyses had shown that the oxalate equivalent of 30 micrograms of calcium could be titrated with an error of plus or minus 0.2 per cent.

One day after the last injections had been given the treated animals and the nontreated controls were killed. The left femur was taken out and fixed in solution of formaldehyde U. S. P. diluted 1 to 10; it was then sawed into an upper and a lower part and decalcified in 5 per cent nitric acid; the pieces were then split lengthwise, the distal end in a sagittal, the proximal end in a frontal plane. Thus a full view of the epiphysis, the metaphysis and the diaphysis was obtained. The tissues were embedded in paraffin, sectioned and stained with hematoxylin and eosin.

OBSERVATIONS ON WEIGHT, HEMOGLOBIN AND PLASMA

Weight.—The initial and final weights can be seen from columns 4 and 5 of the table. The mean final weight of the nontreated controls (1 to 5) was 585 Gm. The mean final weight of 2 bled cockerels killed (19 and 20) four days subsequent

3. The Purina starting mash contains: dehydrated alfalfa meal, an oil containing vitamins A and D, yellow corn meal, meat scrap, oat middlings, Ribolac, mineralized salt, soybean oil meal, wheat bran, wheat germ, ground wheat, fish meal and dried whey.

The Purina growing mash contains: alfalfa meal, ground barley, steamed bone meal, calcium carbonate, an oil containing vitamins A and D, yellow corn meal, ground Kafir-Milo, fish meal, meat scrap, dried whey, Ribolac, mineralized salt, soybean oil meal, soybean meal special, ground wheat and fish solubles.

The chemical analysis and vitamin analysis of these mashes shows:

Protein, %	24.08	19.95	Phosphorus, %	0.95	0.66
Fat, %	3.43	3.92	Riboflavin, parts per mil-		
Fiber, %	5.18	4.47	lion	5.00	4.60
Ash, %	11.44	6.41	Vitamin A equivalent, U.		
Calcium, %	2.38	1.22	S. P. units per gram..	8.00	5.60

3a. The alpha estradiol benzoate (Progynon B) was supplied by the Schering Corporation on the recommendation of Mr. L. H. Cramblet.

4. Evelyn, K. A.: J. Biol. Chem. **115**:63, 1936.

5. Salomon, K.; Gabrio, B. W., and Smith, G. F.: Arch. Biochem. **11**:433, 1946.

TABLE 1.—Experiments on Cockerels

	Number	Duration of Experiment, Days	Amount of Estradiol Benzoate Injected, Mg.	Weight, Gm.		Hemoglobin, Gm. per 100 Cc.		Total Amount of Blood Withdrawn, Cc.	Interval Between Last Bleeding, and Death, Days	Plasma Calcium, Mg. per 100 Cc.		Skeletal Reaction	
				Initial	Final	Initial	Final			Initial	Final	Amount of Bone	Fibrosis
Control cockerels	1	37	197	505	7.0	10.36	10.21	+	0
	2	37	215	600	6.8	11.02	9.84	+	0
	3	40	190	550	7.8	13.45	13.77	+	0
	4	40	193	500	8.0	10.15	10.46	+	0
	5	58	219	705	7.9	7.9	8.91	10.21	+	(+)
Bled cockerels	19	37	210	495	9.5	6.3	74.0	4	10.21	11.02	±	(+)
	20	37	215	190	8.0	5.2	72.0	4	8.91	8.82	±	(+)
	21	40	208	530	7.8	6.3	80.0	14	9.72	11.77	+	+
	23	58	194	530	7.0	6.9	82.5	23	11.50	11.65	+	+
	26	58	197	510	8.6	5.5	83.5	10	10.26	8.91	+	+
	27	58	222	690	9.2	7.5	91.0	10	11.34	11.4	±	0
	7	37	0.84	173	510	8.0	7.94	9.88	±	(+)
Estrogen-treated cockerels	8	37	0.84	200	430	7.5	12.50	10.58	+	0
	9	37	0.84	203	450	7.1	10.37	12.64	±	(+)
	10	40	2.28	175	550	6.8	11.99	12.15	+	+
	11	40	2.28	188	535	7.0	12.15	13.45	+	0
	14	58	3.36	161	565	8.2	7.7	11.31	8.10	±	+
	15	58	3.36	224	630	8.3	7.1	9.40	8.22	±	0
	17	58	3.36	177	625	7.9	6.1	10.04	10.21	+	+
	40	37	0.84	188	475	7.8	4.9	78.0	4	11.76	13.93	(+)	0
Cockerels bled and treated with estrogen	41	37	0.84	191	450	8.4	4.0	73.5	4	8.18	11.03	(+)	0
	29	49	2.28	188	505	8.8	7.6	76.5	14	9.91	13.28	+	(+)
	30	49	2.28	195	455	6.9	5.9	75.0	14	9.23	11.31	±	(+)
	31	40	2.28	197	480	9.8	4.6	59.0	14	9.58	9.60	±	0
	32	40	2.28	204	570	7.2	0.7	73.0	14	8.97	8.82	+	+
	34	58	3.36	220	710	8.9	7.5	82.0	23	9.54	10.53	+	+
	35	58	3.36	215	750	7.6	6.4	92.5	8	11.02	10.69	+	+
	37	58	3.36	226	620	7.9	7.3	88.5	8	9.88	10.69	+	+
	38	58	3.36	218	600	8.6	6.5	80.0	8	9.56	9.56	+	+
	81	58	3.36	305	560	7.0	7.0	80.0	8	10.50	10.21	+	0

to the last bleeding was 492.5 Gm. The weights of the remaining cockerels bled ten days after the last withdrawal of blood and later did not differ from those of the nontreated controls. The mean final weight of the cockerels receiving the estrogen was 554 Gm., and that of the cockerels bled and treated with the estrogen, 561 Gm. The weights of the last two groups were thus only slightly lower than that of the nontreated controls (585 Gm.).

Hemoglobin.—The initial and final hemoglobin values are given in columns 6 and 7, and the total amount of blood withdrawn is shown in column 8. The fluctuation of the hemoglobin level varied in different birds. The maximum amount of blood which can be withdrawn from 30 day old cockerels without producing significant anemia had been determined by an earlier experiment on 10 birds, but in spite of this precaution anemia did develop in a few of the birds toward the end of the bleeding period. In no case was the anemia present for more than four to five days, and in the majority of the birds the hemoglobin level remained fairly constant throughout the experiment.

Plasma.—The initial and final values for calcium are given in columns 10 and 11. No intermediate determinations were made, since in a preliminary experiment on 10 cockerels of the same strain and age and treated in the same way the serum calcium was found to be constant at ten day intervals. In normal birds the plasma calcium fluctuated between 8.91 and 13.77 mg., with a mean of 10.83 mg., per hundred cubic centimeters; the calcium values of the bled birds ranged between 8.82 and 13.77 mg., with a mean of 10.62 mg., per hundred cubic centimeters. For birds treated with estradiol benzoate the calcium values were as follows: between 7.94 and 13.45 mg., with a mean of 10.7 mg., per hundred cubic centimeters for those receiving 0.84 mg. of estradiol benzoate, 12.4 mg. after administration of 2.28 mg., and 9.55 mg. after injection of 3.36 mg. of the estrogen. All these values are within normal limits, and there was apparently no correlation between the slight variations in the plasma calcium and the amount of estradiol benzoate injected; the highest value of calcium, 13.45 mg., was present after administration of 2.28 mg. of estradiol benzoate (no. 11), and a low value of calcium, 8.10 mg., was found after injection of 3.36 mg. of estradiol benzoate (no. 14). Similarly, birds which were bled and treated with this estrogen failed to show deviations from normal levels, the values ranging between 8.18 and 13.93 mg. per hundred cubic centimeters.

Distinct lipemia was observed in the plasma of all birds treated with 3.36 mg. of estradiol benzoate. It was manifested by an intense yellow color and a high viscosity, and the plasma exhibited a fatty layer on standing.

HISTOLOGIC OBSERVATIONS

Normal Cockerels (fig. 1 A).—Three layers of cartilage could be distinguished in the growth zones: (1) the articular layer, composed of resting cartilage cells embedded in abundant eosinophilic matrix; (2) the proliferating layer, containing many more and larger cells, separated from one another by thin strips of basophilic ground substance and showing columnar arrangement; (3) the layer of vacuolated hypertrophic cartilage cells surrounded by small amounts of calcifying chondromucoid matrix. The hypertrophic cartilage cells were corroded by metaphyseal capillaries, which often closely approached but actually did not enter the proliferating cartilage. They were accompanied by loose connective tissue and some osteoblasts. Between the advancing vessels, broad pegs of cartilage reached into the metaphysis and were eroded by the marrow. The cartilage was covered



Fig. 1.—*A*, section of the epiphysis and the metaphysis of the upper end of the femur of an untreated 88 day old cockerel; $\times 50.5$. Note the hypertrophic cartilage eroded by marrow and separated from the latter by a thin osseous lamella. The trabeculae are thin. The marrow is hemopoietic.

B, section of the epiphysis and the metaphysis of the upper end of the femur of an 88 day old cockerel which had been bled since the age of 30 days and killed ten days after the last bleeding; $\times 50.5$. The bony spicules are more numerous and thicker than in *A* and are surrounded by a layer of fibrous tissue.

by a layer of bone, which was thin and discontinuous proximally but which increased in thickness farther distally. In the metaphysis, bony spicules were present, which here and there still contained unopened cartilage cells. These disappeared as the matrix between them calcified. More distally the spicules were covered by osteoblasts and surrounded by a narrow band of loose connective tissue. The marrow was otherwise hemopoietic. The shaft was composed of bone containing small osteocytes and vascular channels filled with loose connective tissue. Nearer the epiphysis the bone was less densely knitted and the channels were wider. The endosteum was discontinuous and thin. Just below the epiphysis the periosteum was thick; it extended into the bone and contained proliferating connective tissue with many multinucleated giant cells. Farther down, the shaft showed a broad transverse osseous protuberance reaching into the cavity.

Bled Cockerels (fig. 1 B).—At no time did the withdrawal of blood affect the cartilage. The number and the size of the cartilage cells and the consistency of the matrix were the same as in the normal controls. The condition of the bone and of the marrow of the metaphysis did not depend on the amount of blood withdrawn or on the length of the period during which the birds had been bled. There were, however, changes varying in degree with the length of the interval from the last bleeding to the time at which the cockerel was killed. In birds killed four days after the last bleeding, the metaphysial spicules were thin and long, and the marrow was congested and composed of hemopoietic tissue. In those killed ten days after the last bleeding the trabeculae, as well as the shaft, were thickened and dense and were surrounded by a layer of loose connective tissue. Both bone and fibrous tissue had increased in amount and density in birds killed fourteen and twenty-three days after the last withdrawal of blood. Farther distally in the diaphysis the marrow was congested and cellular. The shaft contained, besides large amounts of connective tissue, hemopoietic foci.

Cockerels Treated with Estradiol Benzoate (fig. 2).—The hypertrophic cartilage cells were decreased in number and size after seven days of treatment, and this effect was even more pronounced after nineteen and after twenty-eight days of treatment. The advance elements of the marrow consisted not of capillaries as usually but of a poorly vascularized connective tissue, with numerous osteoblasts often forming several continuous layers along the edge of the hypertrophic cartilage. The bony lamella deposited was thicker than that in normal animals and reached high up along the pegs of hypertrophic cartilage. The breakdown of the cartilage was less complete than usual. More cells were left uneroded and were incorporated in newly formed spicules, which thus were thicker than they would be normally and not infrequently interlaced with one another. The metaphysial stroma and that near the transverse protuberance became more abundant as the number of injections of the estrogen increased. Dense fibrous tissue surrounded the thickened and interlaced trabeculae. The fibers were arranged in bundles, new organic matrix appeared, and small preosseous spicules were formed. These increased in thickness and density farther distally, toward the diaphysis, took up calcium and thus were transformed into cancellous bone. The compact bone of the shaft became thicker and contained enlarged vascular channels filled with increasing amounts of connective tissue. The periosteum was likewise thickened and poorly vascularized, and there was increased deposition of bone. The endosteum was composed of a thick layer of connective tissue and numerous osteoblasts and was particularly abundant around the transverse protuberance. From here the newly formed fibro-osteoid tissue encroached on the metaphysis, linking up with the trabecular network therein. Toward the center of the diaphysial cavity these changes decreased, and hemopoietic marrow was present in all the birds.

Cockerels Bled and Subsequently Treated with the Estrogen (fig. 3).—The cartilage did not show any changes as compared with that of the nonbled birds receiving estradiol benzoate. After one week of injections the metaphysial spicules were numerous and long but thin. At the later stages there were many interlacing trabeculae showing cores of cartilage and surrounded by dense connective tissue. Moreover, bony substance formed apparently from the connective tissue. In the birds in which the process was most advanced, bone formation and myelo-

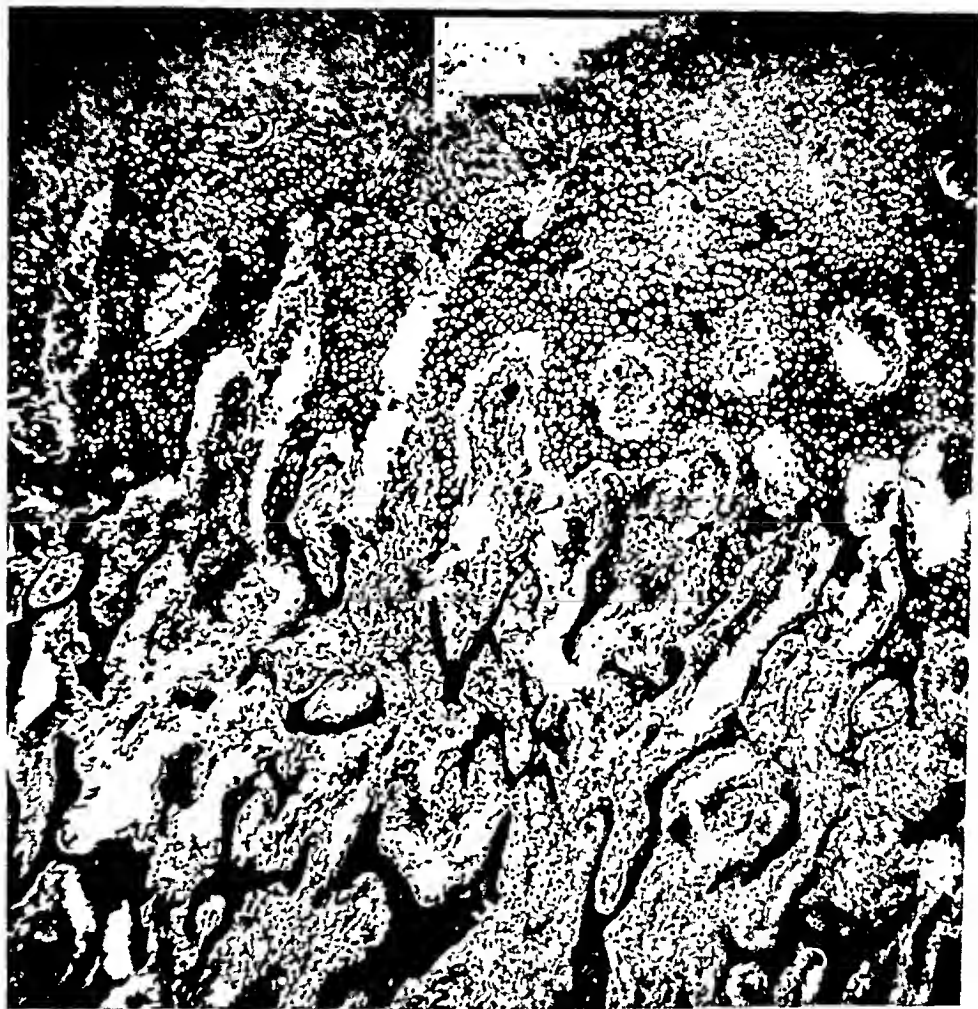


Fig. 2.—Section of the epiphysis and the metaphysis of the upper end of the femur of an 88 day old cockerel which had received 3.36 mg. of estradiol benzoate over a period of four weeks; $\times 52$. Pegs of cartilage reach into the metaphysis. A moderate increase in the amount of bone and fibrous tissue is seen.

fibrosis exceeded those seen under the influence of the estrogen alone. However, there were islands of hemopoietic marrow scattered throughout the metaphysis. They were found even close to the hypertrophic cartilage crowded in between the layers of osteoblasts. The bone of the shaft contained small osteocytes and prominent cement lines. The vascular channels became wider as the bleeding and the injections of the estrogen were continued; but, whereas in nonbled cockerels treated with the estrogen the vascular channels contained only fibrous tissue,

there were many foci of hemopoiesis in the birds that had been bled prior to and during the estrogenic treatment. The endosteum and the periosteum resembled those seen in birds receiving the estrogen alone.

COMMENT

In normal, sexually immature cockerels, the plasma calcium ranged between 8.91 mg. and 13.77 mg. per hundred cubic centimeters. Repeated withdrawals of blood did not affect these levels. Cockerels of the same age

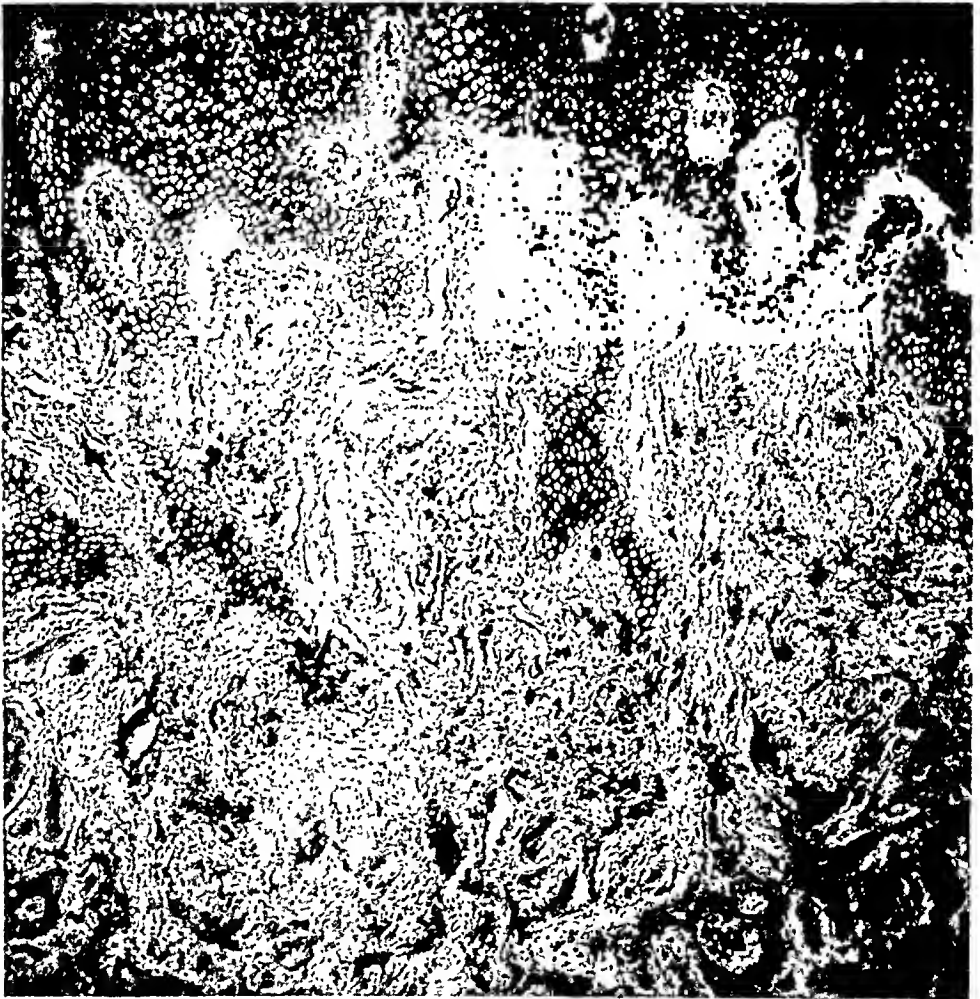


Fig. 3.—Section of the epiphysis and the metaphysis of the upper end of the femur of an 88 day old cockerel which had been bled since the age of 30 days and had received 3.36 mg. of estradiol benzoate over a period of four weeks and which was killed eight days after the last bleeding; $\times 52$. Large amounts of cartilage are uneroded and incorporated into bony trabeculae. More bone and more fibrous tissue are seen than in figure 2.

receiving 0.84 to 3.36 mg. of estradiol benzoate over periods of from one to four weeks likewise showed plasma calcium levels within normal limits. In this respect the cockerels differed from male pigeons, which during the nonbreeding season and after castration showed an increase in serum cal-

cium following administration of an estrogen.⁶ It is possible that an age factor operates in the reaction of the serum calcium to estrogens and that it is responsible for differences in immature birds, on one hand, and mature cockerels, on the other. As in the birds not given injections, bleeding did not change the serum calcium levels in the treated cockerels.

The results of the histologic examination can be summarized as follows: Withdrawal of blood did not affect the proliferation and the hypertrophy of the cartilage. In the metaphysis, however, there were increased fibrosis and formation of bone, increasing with lengthening of the interval between the last bleeding and the date of killing. In columns 12 and 13 of the table the degrees of the metaphysial and endosteal changes are demonstrated in a schematic way by the symbols + to + + + +, + for bone and 0 for fibrous tissue designating conditions found in untreated animals; + + indicates a slight, + + + a moderate and + + + + a striking reaction. There was no correlation between the histologic changes and either the hemoglobin or the calcium levels present at the time of autopsy; three almost identical hemoglobin contents of 6.3, 6.5 and 6.9 gm. (cockerels 19, 21 and 23) were associated with histologic reactions of low or moderate degree, respectively, and with a plasma calcium level of about 11 mg. per hundred cubic centimeters seen in three birds (19, 23, and 27), one moderate and two slight histologic reactions were recorded. The lack of correlation between the calcium levels and the skeletal changes seems especially interesting since in pigeons after the injection of estrogenic substances high calcium levels are due to elevation of the nonultrafiltrable calcium fraction.⁷ The higher demand of the ossifying tissues for calcium may possibly be satisfied by increased turnover of the circulating calcium rather than by elevation of the blood calcium level. Moreover, the bleeding experiments support the view that local factors may be active in ossification and in the production of myelofibrosis.

Estrogen decreased the proliferation and the hypertrophy of the growing cartilage and the breaking down of the cartilage by the marrow, effects similar to those seen in small rodents after treatment with estrogen.^{2a} Moreover, in the cockerels there were myelofibrosis and bone formation accompanied by increased resorptive processes.

The skeletal response to the administration of estradiol benzoate varied considerably in degree in different birds. Some, even after two weeks of treatment, showed hardly any response, whereas in others the reaction was impressive. The variations of the histologic picture were much more conspicuous than the slight fluctuations of the serum calcium level. The immature cockerel thus reacted differently from the mature

6. Bloom, W. A.; McLean, F. C., and Bloom, W.: *Anat. Rec.* **83**:99, 1942.

7. McDonald, M. R., and Riddle, O.: *J. Biol. Chem.* **159**:445, 1945.

cock, in which marked hyperealcemia and extensive hyperossification of the long bones have been observed.⁸

Our findings in immature cockerels are then, on the whole, comparable to those obtained in castrated pigeons and in male pigeons treated with estrogen during the season in which the testicles are resting.⁹ It is thus possible that in the immature cockerel the absence of active testicles is responsible for the fact that the skeletal effects of estrogen are comparatively slight. However, the presence or the absence of active testicles does not fully explain the variations of the estrogenic effects in birds. Immature white Leghorn cockerels showed a more vigorous response to the administration of estrogen than old cocks and fully grown capons,^{2c} a phenomenon also observed in mice⁹ and rats.^{2b} These variations again suggest the presence of an age factor which takes part in determining the reaction to estrogen. There is no synergistic action of androgen and estrogen in sparrows¹⁰ and in certain strains of mice.¹¹

Bleeding previous to and during the administration of estradiol benzoate did not alter the response of the cartilage to the estrogen. It did, however, modify the reaction of the metaphysial and diaphysial tissues in some of the animals treated for two or three weeks. There was no decrease in the myelofibrosis or the ossification as had been expected, but rather a tendency of these processes to increase beyond the degree seen after treatment with estrogen alone. The summation effect might have been even more pronounced, had the interval between the last bleeding and the killing been equally long in the groups receiving the estrogen and in the bled animals not receiving it. In the former the interval was only eight days, at which time the changes in the bled group had not yet reached their peak. The observations indicate that it is possible to modify the effect of estrogen by altering the substratum on which it acts.

SUMMARY

Injections of 0.84, 2.28 and 3.36 mg. of estradiol benzoate failed to raise the plasma calcium level of sexually immature cockerels above the normal upper limits of variation. The estrogen caused inhibition of growth and of breakdown of the epiphysial cartilage and produced myelofibrosis and hyperossification of the metaphyses and the areas close to the endosteal surfaces of the shaft. Cockerels that had been bled prior to and during the administration of the estrogen showed a tendency toward increased myelofibrosis and bone formation.

8. Landauer, W.; Pfeiffer, C. A.; Gardner, W. U., and Man, E. B.: *Proc. Soc. Exper. Biol. & Med.* **41**:80, 1939.

9. Silberberg, M., and Silberberg, R.: *Anat. Rec.* **80**:347, 1941.

10. Pfeiffer, C. A.: *Anat. Rec.* **94**:362, 1946.

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Case Reports

PRIMARY MYXOMA OF THE LEFT VENTRICLE WITH EMBOLIC OCCLUSION OF THE ABDOMINAL AORTA AND RENAL ARTERIES

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PRIMARY tumor of the heart is sufficiently rare to warrant the recording of new instances, particularly those exhibiting important complications.

REPORT OF A CASE

M. S., a 10 year old white girl, entered the Portland Sanitarium and Hospital, Nov. 13, 1940, with the following history:

She had whooping cough in 1934 and measles in 1937. There were recurrent episodes of tonsillitis, with one severe attack in 1934. Pains first appeared in the joints in 1935 and have recurred yearly, especially during cold and damp weather. The pains were sometimes localized in the knees and at other times in the calves of the legs; they were not accompanied by swelling of the joints. The mother states that the first knowledge of any heart trouble came in 1936.

The present illness began one week prior to the patient's entering the hospital, when there was complaint of aching in the muscles of the calves. November 11 she awakened at night with vomiting and diarrhea, complained of pains in the legs and had difficulty in walking. On getting up her legs seemed to cave beneath her. During the afternoon of November 12, convulsions occurred at frequent intervals, and her temperature rose to 101 F. November 13, the convulsive seizures were still occurring, and the patient became completely stuporous. A typical seizure began with slight trembling jerkiness of the left wrist, while the eyes deviated to the right and a coarse nystagmus was directed to the left. After fifteen to twenty seconds this shifted, and similar jerkiness was noted in the right wrist, a little larger and more extensive than that in the left, together with conjugate deviation of the eyes to the left and coarse nystagmic jerks to the right. Later there were some jerking movements of the whole right arm and leg. The entire episode lasted for several minutes.

The child was well developed and well nourished and in a completely stuporous condition. The skin was fair and without evidence of petechial hemorrhages. There was no stiffness of the neck and no facial asymmetry. The conjunctivas were clear; the pupils measured 4.5 mm. and were round and equal; the response to light was prompt and adequate. The optic disk showed no abnormality; the physiologic cup was well defined; the vessels were normal; there were no hemorrhages. The tonsils were absent; the pharynx was boggy and reddened. The tympanic membranes exhibited a little diffuse redness but no bulging.

The tonus of the arms and the legs was normal, with movement possible in both arms and legs. The deep reflexes of the arms and the legs were active; there was a bilateral positive Babinski reflex; Kernig's sign was not shown.

The lungs were clear to auscultation and percussion.

Examination of the heart revealed sinus tachycardia, with a systolic thrill at the apex. The heart was slightly enlarged to the left without filling of the waist.

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There was a 1 to 2 plus lower sternal thrust. The mitral first sound was followed by a rough systolic murmur, transmitted well into the axilla and up over the precordium. There was a loud systolic murmur at the aortic area, different in character from the mitral systolic. The aortic second sound was well heard, not accentuated and louder than the pulmonary second sound; it was followed by a definite soft diastolic blow, which could also be heard at the third left interspace and was transmitted down to the left sternal border.

The abdomen was not tender and was without masses and without palpable organs.

The impression was old rheumatic heart disease, acute pharyngitis with gastroenteritis, and serous meningitis.

The spinal fluid pressure was grossly increased; the fluid was clear. The total protein content was 47 mg. per hundred cubic centimeters; the sugar content, 87 mg.; the chloride content, 759 mg., and the cell count, 0.

The blood showed 26,000 leukocytes per cubic millimeter, 82 per cent of which were polymorphonuclear leukocytes and 8 per cent staff cells. The red cell sedimentation rate was 15 mm. in forty-five minutes.

As the day (November 13) advanced the clinical picture changed considerably. The convulsive seizures stopped after the administration of small doses of phenobarbital; marked facial edema developed; no urine was passed over a twenty-four hour period, and catheterization yielded only a few cubic centimeters of very bloody urine with some pus. The blood pressure was 150 systolic and 84 diastolic. No petechiae appeared anywhere.

The clinical impression then was: acute rheumatic fever; acute glomerulonephritis with anuria; old rheumatic heart disease with mitral insufficiency (and probably stenosis); aortic insufficiency and stenosis. It was not thought at that time that subacute bacterial or acute bacterial endocarditis was present.

Under continued sedation the convulsions did not recur; the almost complete anuria continued, as did the facial edema. The blood urea level was first 107 mg. and then, later, 170 mg. per hundred cubic centimeters; the systolic blood pressure climbed to 170 mm. of mercury. A second study of the blood gave 35,000 leukocytes, with polymorphonuclears 82 per cent and staff cells 8 per cent; the sedimentation rate was 11 mm. in sixty-seven minutes.

The impression was the same as previously, although now embolism with blocking of the renal arteries and renal infarction was considered.

On the last day of life a new phenomenon developed. Following the long apical systolic sound there was a loud snap, "like a shot," as if the valves had suddenly ruptured. This came not at the time of the mitral first sound but after the mitral systolic murmur. It would last for a few beats and then disappear. The sound was so loud that it could be heard several inches away from the chest. In view of the lack of constancy and timing the sound was not believed to be derived from the heart but to be rather some anomalous extracardiac sound. The child died on November 18, five days after admission.

The final diagnosis was: old and active recurrent rheumatic fever; old rheumatic heart disease with aortic stenosis and insufficiency, mitral insufficiency and probably stenosis; acute fulminating glomerulonephritis with uremia.

Autopsy.—Permission to remove the brain was denied, and for this reason the cause of the convulsions, if located in the brain, remains obscure. Since the only pathologic processes were confined to the cardiovascular system and the kidneys, only the positive findings need be given.

(a) Heart: This organ had a transverse diameter of 11 cm. Aside from pericardial and subepicardial petechial hemorrhages the surface of the heart was unchanged. The left ventricle formed the entire apex, and its left border was prominent and rounded. The right side of the heart, together with the great vessels entering and leaving it, was unchanged. The foramen ovale was closed. The wall of the left ventricle measured 2 to 2.5 cm. in thickness, and its muscle was firm and of coarse texture. The anterior leaflet of the mitral valve appeared thickened and whitened along the line of closure but was without active vegetations; the chordae tendineae of this valve were thickened but not appreciably shortened. While stenosis was absent, it was considered possible that an insufficiency may have existed during life. The left atrial appendage was free from thrombosis. While all cusps of the aortic valve were thick enough to be white and opaque, there was no apparent stenosis. The left posterior leaflet exhibited two small fenestrations near one of its commissures. No vegetations were present. Just above the aortic valve were three transversely directed atheromatous streaks involving the aortic intima.

Practically filling the left ventricle and extending upward to the level of the commissure of the aortic valve were multiple polypoid masses, the largest of which measured 5 by 3 by 2 cm. (fig. 1, at the left). This and others of smaller size appeared to be intimately attached to the endocardium over an area of approximately 1.5 to 2 cm., at a point a little below the insertion of the posterior papillary muscle of the mitral valve. Much of the main mass was jelly-like, smooth of surface and faintly yellow, although at the base the color darkened and the texture became firmer. A second soft smooth-surfaced yellow polyp, measuring 2.5 by 1 by 0.8 cm., was attached by a pedicle 3 mm. in diameter; a third, of similar appearance, measured 2.5 by 1.5 by 1.5 cm.; a fourth, nestled in the apex of the ventricle, had dimensions of 2 by 2 by 1.5 cm. and differed from all the others in being dark red throughout. All polyps appeared to have a common attachment along with the major polyp extending through the orifice of the aortic valve. However, a sixth mass, measuring 2 by 0.5 by 0.5 cm., sprang from the endocardium at the level of the free margin of the posterior leaflet of the mitral valve. Finally, on the left border of the base of the largest polyp and very near the pedicles of two others, was a slightly elevated, roughened surface, 5 mm. in diameter, which appeared to represent the point from which something had broken off. No thrombi occurred over the roughened area or anywhere else in the left ventricle.

(b) Abdominal Aorta and Kidneys: Lodged in the aorta at a point slightly caudal to the ostium of the celiac artery and extending distal to the ostiums of the renal arteries was a shiny-surfaced, soft and wet-appearing mass measuring 5.3 by 1.3 by 1.5 cm. (fig. 1, at the right). The distal end was somewhat pointed, whereas the proximal end was rough, ragged and apparently torn. The embolus was lightly attached to the aortic intima at a number of points, and when it was removed the vessel's surface at these places was slightly roughened. The mass was spongy, yellow, wet, and bore a close resemblance to the polyps in the heart. There was a little space between the embolus and the ostium of the superior mesenteric artery.

The main renal arteries were patent near the kidneys, but next to the aorta they were occluded by very soft pale masses directly attached to the mass filling the aorta. The kidneys, after the renal capsules had been stripped off, proved to be mottled, being alternately yellowish white to brown to black. When they were sectioned, extensive anemic infarction was seen to involve the cortex, often extending into the columns of Bertini. By contrast, the pyramids, especially those of



Fig. 1.—Visible along the septal aspect of the left ventricle are five smooth-surfaced polypoid masses having a common origin near the insertion of the posterior papillary muscle of the mitral valve. The largest projects through the aortic valve, the cusps of which are scarred as a result of healed rheumatic valvulitis.

Lodged in the abdominal aorta is a large tumor embolus which, being soft, extruded into the renal arteries, with resultant bilateral renal infarction and acute renal insufficiency.

Fig. 2.—High power photomicrograph illustrating the cell-poor structure of the tumors. Both the homogeneous and the more vacuolated portions give the staining reaction of mucin.

the left kidney, were almost black. Numerous petechial and ecchymotic hemorrhages involved the renal pelves.

(c) Lungs: These showed hyperemia, focal atelectasis and edema.

Microscopic Examination.—Sections from different parts of the main polypoid mass and sections of the dark red polyp, as well as some including the point of attachment of these bodies, revealed a structure poor in cells and consisting mostly of a homogeneous or stringy substance staining all the way from blue through bluish pink to red with hematoxylin-eosin. In the stringy substance were many crevices or larger openings, often bordered by ovoid or rounded cells of considerable size with deeply staining nuclei. The different polyps appeared to be well anchored to the heart wall by capillary vessels, by the homogeneous substance already described and by fibroblasts. The only additional cells within the polyps were neutrophilic polymorphonuclear leukocytes and monocytes except in the very dark tumor, in which the color was explained by the presence of a large hemorrhage. Endothelial cells could not be demonstrated over the external surface of any of the tumors, despite its smooth and shiny appearance. Despite the apparent lack of endocardium, there were no thrombic deposits anywhere over the various polyps.

Sections stained for mucin with thionine displayed varying shades of pink to red in much of the wavy bandlike material and the other more homogeneous or vacuolated intercellular substance, a staining reaction compatible with mucin. The nuclei of all cells were deep blue, while the cytoplasm of all known cells was either faintly blue or unstained. The cytoplasm of certain rather infrequently encountered cells with ovoid to elongated nuclei, containing scattered chromatin granules, appeared pink to red and extended out in a stringy and pointed fashion, often on only one side of the nucleus. Thus the cytoplasm of the cells just mentioned may be said to have had the same staining reaction as the intercellular substance, which was that of mucin.

The structure of the aortic embolus and of the extensions into the renal arteries corresponded to that of the tumors in the heart.

There was no microscopic evidence of active rheumatic endocarditis or myocarditis.

COMMENT

A summarization of the literature dealing with primary cardiac tumors is not required at this time. The subject has been well covered in the reviews of Lisa, Hirschhorn and Hart¹ and Yater.²

The unusual features of our case were (1) the left ventricular origin of the tumor and (2) the embolic occlusion of the abdominal aorta and renal arteries by a large tumor embolus. Embolic phenomena associated with these relatively friable growths are surprisingly rare and when present are most often found in the cerebral arteries. Often, as in our case, there occur relative stenosis and insufficiency of a valve due to the fact that the pedunculated tumor mass projects through the valvular orifice. A left ventricular origin of myxoma, while infrequent, nevertheless stands next in incidence to a left atrial origin. The question arises as to whether the tumor was true myxoma or an organ-

1. Lisa, J. R.; Hirschhorn, L., and Hart, C. A.: Arch. Int. Med. 67:91, 1941.

2. Yater, W. M.: Arch. Int. Med. 48:626, 1931.

izing thrombus. On the basis of the multiple polypoid character and the staining reaction with thionine, the weight of evidence is in favor of myxoma.

SUMMARY

An unusual multipolypoid primary tumor diagnosed as myxoma originated at the apex of the left ventricle. As a result of embolization of the abdominal aorta there were first the symptoms of acute intestinal obstruction. These were followed by bilateral renal infarction and fatal acute renal insufficiency. The temporary character of the symptoms of intestinal obstruction is explained by the soft and compressible character of the embolus, which allowed it to sink downward, freeing the ostium of the superior mesenteric artery. The same qualities allowed the embolus to extrude into the ostiums of the renal arteries. The initial episode of convulsions, stupor and nystagmus may have been due to cerebral embolism but denial of the privilege of removing the brain leaves this phase of the clinical course unexplained. The striking change in the heart sounds during the latter part of the child's life may have coincided with the breaking off of the tumor embolus.

PULMONARY ADENOMATOSIS COMPLICATED BY LOBAR PNEUMONIA

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PRI-MARY multiple tumors of the lungs composed of epithelial cells lining pulmonary alveoli and without extrapulmonary metastases are rare. At least 9 cases have been recorded in the literature. Arranged in chronologic order, they are the cases of Helly¹ (1907), Oberndorfer,² Bonne,³ Richardson,⁴ Sims,⁵ Bell,⁶ Taft and Nickerson⁷ (2 cases) and Wood and Pierson.⁸ Other cases which may possibly belong in the same group have been reviewed by Neubuerger and Geever⁹ in a discussion of alveolar cell tumors.

Because of the rarity of this neoplasm, we wish to report another case, in which there was terminal lobar pneumonia.

REPORT OF A CASE

J. H., a 56 year old white widow, was first admitted to the Second Medical Division of Bellevue Hospital on May 17, 1940, with the chief complaint of weakness of the right side of the body for twenty-four hours. The Wassermann test of the blood and the spinal fluid showed a 4 plus reaction in each instance. A roentgenogram of the chest disclosed a "fibrotic patch in the second right interspace in the axillary line." There was a rapid return of function in the extremities following therapy with malaria and oxophenarsine hydrochloride. During her follow-up in the clinic, the results of tests of the blood and the spinal fluid reverted toward normal. She was discharged July 15, 1940.

From Jan. 8 to May 1, 1945 the patient was again in the hospital because of fever, pain in the right anterior part of the chest and bloody sputum. Four months prior to admission she noted the onset of localized wheezing in the right upper part of the chest and had a cough productive of scant mucoid sputum. Two weeks before admission she "caught a cold," which was followed by fever, pleuritic pain, cough and increased sputum.

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1. Helly, K.: *Ztschr. f. Heilk.* **8**:105, 1907.
2. Oberndorfer, S.: *Virchows Arch. f. path. Anat.* **275**:728, 1929.
3. Bonne, C.: *Am. J. Cancer* **35**:491, 1939.
4. Richardson, G. O.: *J. Path. & Bact.* **51**:297, 1940.
5. Sims, J. L.: *Arch. Int. Med.* **71**:403, 1943.
6. Bell, E. T.: *Am. J. Path.* **19**:901, 1943.
7. Taft, E. B., and Nickerson, D. A.: *Am. J. Path.* **20**:395, 1944.
8. Wood, D. A., and Pierson, P. H.: *Am. Rev. Tuberc.* **51**:205, 1945.
9. Neubuerger, K. T., and Geever, E. F.: *Arch. Path.* **33**:551, 1942.

She was acutely ill, moderately dyspneic and slightly cyanotic. The pupils were equal and reacted to light. The trachea was deviated to the right. There were signs of consolidation over the upper lobe of the right lung.

The hemoglobin content was 13.5 Gm.; the white blood cell count, 18,000 per cubic millimeter, with 90 per cent polymorphonuclear leukocytes and 10 per cent lymphocytes. The urine was normal. The Wassermann test of the blood was negative. Repeated examinations of the sputum revealed no acid-fast bacilli. Culture of the sputum showed *Streptococcus viridans* and *Staphylococcus aureus*.

The patient made an immediate clinical response to sulfadiazine. Her temperature became normal on the third day. The sputum gradually decreased in amount and became free of blood; however, after a month she continued to raise a half-cup of mucoid sputum daily. The signs of consolidation in the upper lobe of the right lung persisted and were confirmed by roentgenogram. Two bronchoscopic examinations were done; nothing abnormal was found. Studies with iodized poppyseed oil 40 per cent were unsatisfactory. She received a course of treatment with penicillin, and postural drainage was instituted, without improvement. She was discharged to the chest clinic.

Permission to use the clinical records from the Chest Service of Bellevue Hospital was given by Dr. J. Burns Amberson.

The patient was hospitalized for the third time from Dec. 13 to Dec. 22, 1945. Since the previous discharge she had continued to have a cough productive of small amounts of mucoid sputum, and occasionally she suffered from dyspnea. One day before her admission fever, pleuritic pain and increasing dyspnea developed. There was no palpitation, nocturnal dyspnea, precordial pain or edema.

She was acutely ill, dyspneic and slightly cyanotic. Signs of consolidation were present over the entire right side of the chest. Both lungs were filled with bubbling rales and rhonchi. The heart seemed enlarged to percussion, with sounds obscured by the respiratory din. A roentgenogram of the chest revealed massive consolidation of the right lung, with deviation of the trachea to the right. The hemoglobin content was 11 Gm.; the white blood cell count, 6,000. The urine contained albumin (3 plus) with 25 red blood cells per high power field. The Wassermann test of the blood was negative. Culture of the sputum revealed *Staph. aureus*, *Str. viridans* and diphtheroids. The nonprotein nitrogen was 31 mg. per hundred cubic centimeters.

The patient was treated with a positive pressure oxygen mask, intravenous theophylline ethylenediamine injection, U.S.P., and penicillin and given rapid digitalization. In a day and a half the temperature fell to normal. However, the pulse rate remained about 120 per minute. Initially, the patient improved, and the lungs contained fewer rales. An inconstant expiratory wheeze was heard over the upper lobe of the right lung. The patient required continuous administration of oxygen, became worse, had increased rales in both lungs and died on the tenth day in the hospital.

The clinical diagnosis was lobar pneumonia, pulmonary fibrosis and emphysema, and bronchiectasis.

Necropsy.—The principal abnormal conditions were in the thoracic cavity. The visceral and parietal pleurae over the upper and middle lobes of the right lung were united by dense fibrous adhesions. The lower lobe of the right lung was covered by fibrinous exudate. There were no pleural adhesions over the left lung. Both pleural cavities were free of fluid. The trachea was deviated to the right.

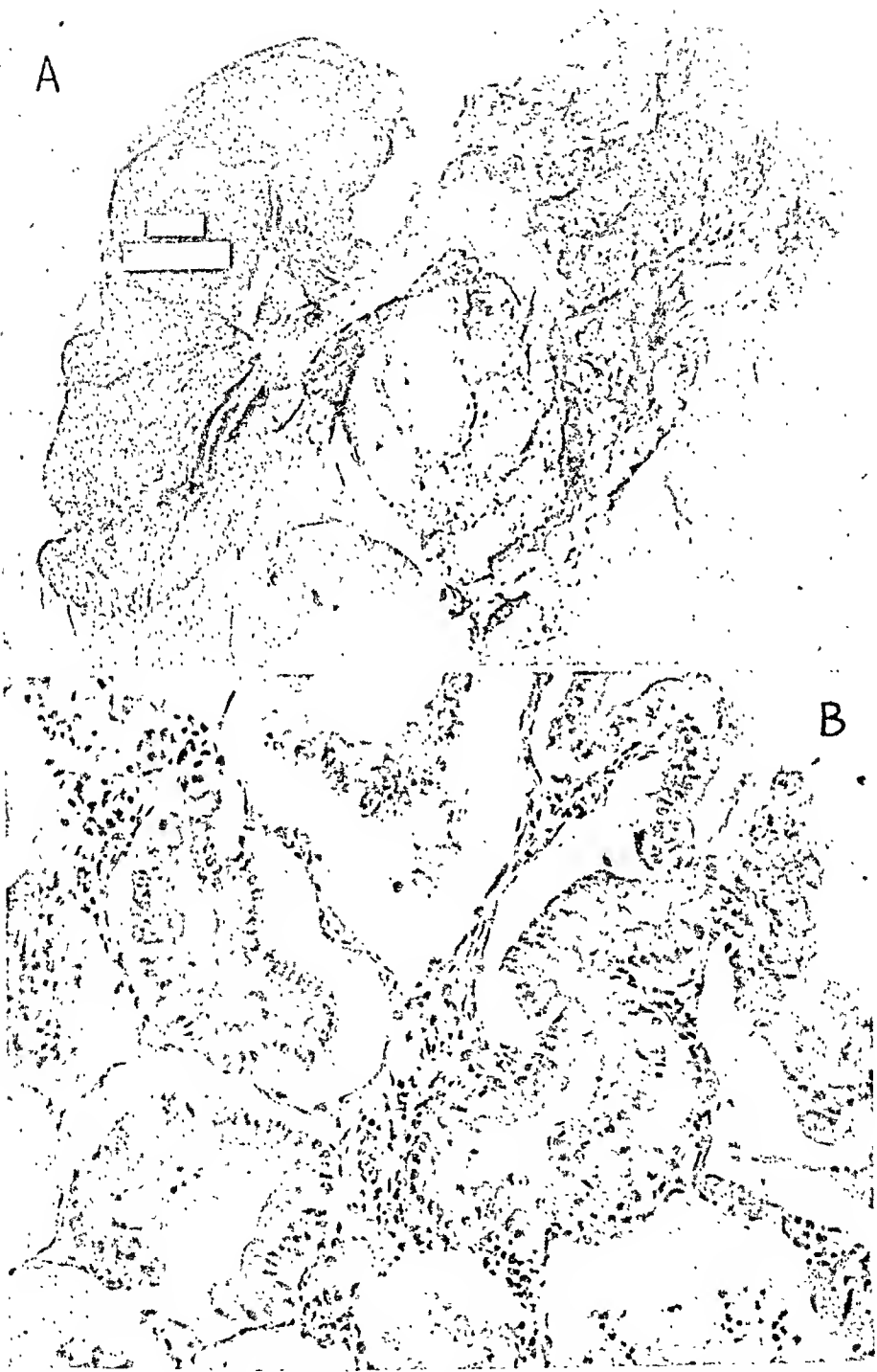


Fig. 1.—*A*, lobar consolidation and fibrinous pleurisy of the lower lobe of the right lung and bronchiectasis and fibrosis of the upper lobe of the right lung. *B*, lining of alveoli by nonciliated columnar epithelial cells forming mucin. From the lower lobe of the right lung; $\times 198$.

The lungs together weighed 2,100 Gm., the right lung constituting about three quarters of the total weight. The upper and middle lobes of the right lung were small, practically airless and reddish gray on section. Their bronchi showed a fusiform dilatation.

The lower lobe of the right lung was large, heavy and consolidated throughout. The parenchyma was yellow-pink, with a moist, gelatinous cut surface. At the apex of the lobe there was necrosis of the parenchyma, with formation of a yellow-green exudate. The gross appearance of the lower lobe of the lung closely resembled the consolidation seen in pneumonia due to Friedländer's bacillus.

The left lung was of normal size, with a smooth gray pleura. On section the parenchyma was pink and crepitant. In the lower lobe of the left lung, about 2 cm. above the diaphragmatic pleura, there was a solitary nodule, 1 cm. in diameter, with a yellow gelatinous cut surface and a fairly firm consistence. This nodule had no demonstrable relationship to the bronchi or the bronchioles.

The bronchi of both lungs contained a small amount of viscous yellow fluid. At no point were the bronchi stenosed or ulcerated.

The peribronchial and tracheobronchial lymph nodes were small, soft and uniformly gray on section.

No similar tumor tissue was found in any other organ.

The spleen weighed 150 Gm.; microscopically, the red pulp was engorged with red blood cells, and there was an increased number of polymorphonuclear leukocytes. The liver showed a mild fatty change. A microscopic adenoma of islet tissue, about five times the size of a normal islet of Langerhans, was present in the pancreas. There was mild bilateral chronic salpingitis. No evidence of syphilis was found at necropsy.

Histologic examination of the lungs revealed a remarkable picture, varying according to the lobe sectioned.

In a section from the lower lobe of the right lung the pleura was engorged and covered by organizing fibrinous exudate. Nearly every alveolus contained fibrinopurulent exudate, and some of the alveolar septums were necrotic. The interlobular septums were edematous and contained neutrophilic leukocytes. Exudate containing epithelial cells was present in the bronchioles, the walls of which were acutely inflamed.

In many scattered foci, varying in diameter from 1 to .3 mm. in the stained preparation, the alveoli contained tall columnar cells that covered the alveolar septums in a single layer. The cells were larger than the columnar cells of the bronchial mucosa. Their nuclei were of moderate size, oval, basally situated in the cells, and had abundant chromatin, finely dispersed. Occasional nuclei had single basophilic nucleoli. The cytoplasm was abundant, homogeneous and eosinophilic. Most of these cells contained droplets of basophilic secretion in the cytoplasm near their free margins. Many alveoli lined by these cells were filled with basophilic secretion. The cells were nonciliated. Mitoses were not seen. There was considerable shrinking of these cells away from alveolar septums; this was considered to be largely an artefact. There was no papillary formation.

A mucicarmine stain demonstrated droplets of mucin within the cells and abundant mucin in the lumens of tumor-line alveoli.

A Wilder-Van Gieson stain for reticulum revealed a few reticulum fibers between the basilar portions of the lining cells in addition to the normal reticulum framework of the alveolar septums. Some of the tumor cells contained argyrophilic granules in the apical portions of the cells; these granules corresponded in location to the droplets of mucin seen with the mucicarmine stain.



Fig. 2.—Wilder-Van Gieson stain for reticulum. No increase in fibrous tissue is present in the alveolar septums. The adjacent interlobular septum is not invaded. $\times 198$.

B, space lined by mucin-secreting epithelial cells in the fibrotic upper lobe of the right lung. $\times 108$.

A Brown-Breu stain revealed numerous and varied organisms in the pneumonic exudate, including gram-positive cocci (some in short chains), rare gram-positive diplococci and numerous short gram-negative bacilli.

Sections from the upper and middle lobes of the right lung revealed extensive fibrosis of the pleura and the parenchyma, with infiltrating neutrophils, lymphocytes and plasma cells. There were dilated bronchioles having a thick epithelial basement membrane and chronically inflamed walls. Also, large spaces were lined by columnar tumor cells and contained mucin and purulent exudate. These spaces lacked the muscle fibers and epithelial basement membrane of bronchioles.

Sections of the main bronchi in the upper and lower lobes of the right lung showed no neoplastic involvement.

A section from the upper lobe of the left lung was free of tumor.

A section of the nodule in the lower lobe of the left lung showed it to be composed of tumor-lined alveoli filled with mucin. The mucin had overflowed into alveoli that were free of tumor.

In none of the sections was it possible to demonstrate that the columnar cells lining alveoli were continuous with the bronchiolar epithelium. The epithelium lining the bronchi and the bronchioles was not hyperplastic. No invasion of blood or lymphatic vessels was seen.

Several sections of peribronchial and tracheobronchial lymph nodes showed no metastases; neither were metastases found in sections of other organs, nor in those of the vertebral marrow.

The anatomic diagnosis included: pulmonary adenomatosis of the upper, middle and lower lobes of the right and the lower lobe of the left lung; bronchiectasis and fibrosis of upper and middle lobes of the right lung; lobar pneumonia, lower lobe of the right lung; fibrinous pleurisy, lower lobe of the right lung; acute splenic tumor; adenoma of an islet of Langerhans; fatty liver, mild; chronic salpingitis.

COMMENT

The clinical course in this case was characterized by two episodes of pneumonia eleven months apart, the second episode ending in death. A roentgenogram taken following the first illness disclosed an unresolved process in the right upper lung field. At necropsy the upper and middle lobes of the right lung were fibrotic and contained tumor. We think it probable that the evolution of the tumor occurred during or even before the onset of the first episode of pneumonia. It seems unlikely that pneumonia contributed to the genesis of the tumor, since at necropsy there was a nodule of tumor in the lower lobe of the left lung, where there was no evidence of inflammation or fibrosis.

Indeed, it seems probable that the multiple tumors, with their abundant production of mucin, predisposed the involved parenchyma to infection. Some experimental evidence may support this hypothesis. Nungester and his co-workers¹⁰ found that in rats an intrabronchial injection of mucin increased the incidence of pneumonia caused by pneumococci injected intrabronchially or intravenously.

10. Nungester, W. J., and Jourdonais, L. F.: *J. Infect. Dis.* **59**:258, 1936. Kempf, A. H., and Nungester, W. J.: *Proc. Soc. Exper. Biol. & Med.* **43**:627, 1940.

The gross resemblance of the lower lobe of the right lung in our case to a lung with Friedländer's pneumonia duplicates a previous observation in 2 cases reported by Taft and Nickerson.⁷

There is no evidence that syphilis contributed to the genesis of this patient's pulmonary disease; a positive serologic test has not been reported in other published cases. The pancreatic islet tumor was apparently silent clinically.

Clinically, this disease simulates a chronic infectious disease of the lungs. Although clinical data are not available in all reported cases, it has been noted that cough was present in all cases in which mention was made of this symptom. Other symptoms usually present are dyspnea and loss of weight. Thoracic pain was described in 2 cases (Bonne³; Sims⁵). Hemoptysis was reported in only 1 case (Wood and Pier-son⁸). The age of the patients has varied from 21 to 79 years; the sexes have been equally represented.

The average duration of the disease appears to be several months. Oberndorfer's² patient, a man aged 21, died after a brief illness, and, in addition to multiple foci of tumor, had extensive hemorrhagic pneumonia. On the other hand, Bell's⁶ patient had symptoms of cough, dyspnea and loss of weight at least two and a half years before death. Most of the patients had pertinent symptoms at least nine months before death.

The clinical course had apparently never been sufficiently typical to make possible a correct diagnosis before pathologic study was possible.

The prognosis of this disease appears to be poor, the patient dying either of superimposed pneumonia or of respiratory insufficiency resulting from the lining of alveoli by tumor cells. In the only patient treated surgically by lobectomy, other lobes were subsequently found to be involved by the tumor.⁸

In the literature a lively discussion had occurred on three points: (1) the origin of the tumor cells; (2) the nature of the tumor, benign or cancerous; (3) the possible relationship of the tumor to an anatomically similar infectious disease of sheep sometimes called "jagziekte."

Origin of the Tumor Cells.—Do they arise in the alveoli or are they derivatives of bronchiolar epithelium? This point is unsettled, since opposing observers are not in agreement even as to the validity of some of the evidence presented. The subject is well reviewed by Geever, Neubuerger and Davis.¹¹

Even the debate whether alveolar lining cells occur in the adult human lung is unsettled. Bell⁶ and Geever, Neubuerger and Davis¹¹ studied the lung as affected by chronic passive congestion and inflammatory condi-

11. Geever, E. F.; Neubuerger, K. T., and Davis, C. L.: *Am. J. Path.* 19:913, 1943.

tions. They observed epithelial proliferation from alveolar lining cells. They concluded that epithelial cells occur in the alveoli of the normal adult human lung but do not form a continuous lining. In their opinion these cells may give rise to tumors. Recently, however, Herbut¹² studied regenerating epithelium lining alveoli in cases of bronchiectasis by serial sections and concluded that the regenerated epithelium was derived from bronchiolar epithelium.

The distribution of the tumor in multiple foci, lining alveoli and without demonstrable continuity with bronchiolar epithelium, argues for an alveolar origin of the growth. This view has been taken by, among others, Bell,⁶ Geever, Neuburger and Davis,¹¹ Taft and Nickerson⁷ and Wood and Pierson.⁸

It appears that in animals similar tumors arise from alveolar cells. Cowdry and Marsh¹³ have studied jagzkiekte in sheep and concluded that the proliferated cells arise through "a metamorphosis of alveolar epithelium." Grady and Stewart¹⁴ produced multiple tumors in the lungs of mice by injecting subcutaneously 1,2,5,6-dibenzanthracene and methylcholanthrene. In sections taken at different intervals following injection, they traced the evolution of epithelial tumors from proliferated cells in the alveolar septums.

The columnar type of tumor cells in human pulmonary adenomatosis, with abundant production of mucin, led Richardson⁴ to favor an origin from bronchiolar epithelium.

Recently, Herbut has denied the existence of tumors derived from alveolar cells. The capacity to line alveoli does not prove the alveolar origin of the cells, he observed, because such lining tendency is seen occasionally in carcinoma proved to be bronchogenic and in adenocarcinoma of the lung secondary to carcinoma of the gallbladder, the colon and the pancreas. He pointed out that overgrowth of the tumor may obscure the point at which it originates from bronchial or bronchiolar epithelium. He also stated that the fact that there are multiple foci in the lungs does not prove a multicentric origin of the tumor, since the tumor may spread within the lung via lymphatics or via bronchial or bronchiolar lumens.

We believe that in our case the tumor was most likely of multicentric origin since there was no demonstrable invasion of lymphatic vessels. The idea that the tumor spreads by bronchiolar lumens is purely hypothetical.

Evidence for Classification of the Tumor as Benign or Cancerous.—In the 9 cases cited by us from the literature the growth was adenoma in the sense that it was composed of columnar cells lining alveoli and

12. Herbut, P. A.: *Am. J. Path.* **20**:911, 1944; *Arch. Path.* **41**:175, 1946.

13. Cowdry, E. V., and Marsh, H.: *J. Exper. Med.* **45**:571, 1927.

14. Grady, H. G., and Stewart, H. L.: *Am. J. Path.* **16**:417, 1940.

having usually the capacity to secrete mucin. There were no metastases even in the regional lymph nodes, whereas bronchogenic carcinoma is known to involve lymph nodes early.

In most of the reported cases the cells have been rather uniform, with rare or no mitoses.

Local invasiveness was observed only in the case reported by Bonne, in which the pleura was involved. Vascular invasion has been absent.

All of these features, in our opinion, favor the view that this is a benign tumor of multicentric origin, aptly termed "pulmonary adenomatosis."

It is true that similar cases have been reported in which metastases were found in lymph nodes or distant organs (Weissmann^{15a}; Briesse^{15b}; other cases cited by Neubuerger and Geever). A review of the histologic descriptions and illustrations of these cases leads us to conclude that in most instances of "alveolar cell" carcinoma the neoplasm is more pleomorphic, with numerous mitoses, nuclei larger in proportion to the cells and often bizarre giant nuclei. However, the resemblance between pulmonary adenomatosis and metastasizing alveolar cell tumor is sufficiently close to suggest the possibility that pulmonary adenomatosis is a potentially malignant tumor.

Resemblance to Jagziekte.—Jagziekte is an infectious disease of sheep, of unknown etiologic agent, occurring in Iceland, South Africa and England. A similar infectious disease, "progressive pneumonia," occurs in sheep in Montana. Cowdry and Marsh¹³ have studied these conditions extensively. The disease lasts several months and is always fatal, death occurring apparently from asphyxia. At necropsy the lungs disclose "chronic catarrhal pneumonia," with many alveoli lined by cuboidal and columnar cells, which often show papillary infolding into alveolar lumens. Mucin formation is not prominent. The tumor is not invasive; in only 1 case, reported by Aynaud (cited by Dungal,¹⁶) have metastases been found in regional lymph nodes. The similarity of the disease to human pulmonary adenomatosis has been mentioned repeatedly. For a fuller discussion, the reader is referred to the articles of Bonne³ and Dungal.¹⁶

No person with pulmonary adenomatosis has ever been proved to have been exposed to diseased sheep, and most cases of human adenomatosis have been reported in localities where the ovine disease is unknown.

Thus, while a symptomatic and anatomic similarity exists between human and ovine adenomatosis, there is no evidence that the two diseases have a common cause.

15. (a) Weissmann, S.: Frankfurt. Ztschr. f. Path. 47:534, 1935. (b) Briesse: Frankfurt. Ztschr. f. Path. 23:48, 1920.

16. Dungal, N.: Proc. Roy. Soc. Med. 31:497, 1938.

SUMMARY

Pulmonary adenomatosis involving four lobes of the lungs has been observed.

It is probable that the multiple tumors, producing abundant mucin, predisposed the lungs to pneumonic infection.

It remains uncertain whether in pulmonary adenomatosis the tumor cells arise from alveolar lining cells or from bronchiolar epithelium.

This disease resembles infectious adenomatosis of sheep, although proof is entirely lacking that the two diseases are related etiologically.

SYSTEMIC INFECTION WITH CRYPTOCOCCUS NEOFORMANS (TORULA
HISTOLYTICA) AND HISTOPLASMA CAPSULATUM IN
THE SAME PATIENT

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Mixed infection with pathogenic fungi is of extreme rarity.

REPORT OF A CASE

B. H., a 12 year old Negro boy, was admitted to the medical service of the University of Virginia Hospital, complaining of weakness and loss of weight of two or three months' duration. The history obtained was meager because of the patient's debility.

He apparently enjoyed good health until approximately three months before admission, when there was an insidious onset of weakness, anorexia, progressive loss of weight and night sweats. Five to six weeks before admission a red, painful swelling developed over the right sternoclavicular joint, which was incised by his local physician one month before admission. A yellow mucoid material was obtained, which was said to be sterile. The incision failed to heal, and the lesion continued to drain. Two weeks before admission the patient noted a nonproductive cough and began to bleed from the gums. All of his symptoms progressed, and during the week prior to admission he became so weak that he could not walk.

When examined by us the patient was extremely emaciated and acutely ill. His temperature was 100.2 F. (38 C.), pulse rate 120, respirations 32 and blood pressure 95 systolic and 40 diastolic. The skin was dry and scaly. The mucous membranes were pale. Several teeth were crusted with blood. There was general lymphadenopathy; the involved nodes were nontender. Over the right clavicle just lateral to the sternum there was an indurated, tender area 4 by 5 cm., in the center of which was an ulcer about 1 cm. in diameter. Thick sanguinous fluid drained from the ulcer. The spleen extended 3 cm. below the costal margin. The liver was firm and extended 8 cm. below the right costal margin. There was no evidence of ascites. Slight edema was present over the shins. The heart and the lungs were essentially normal.

The hemoglobin content (Haden-Hauser method) was 5.25 Gm.; the erythrocyte count, 1,940,000; the leukocyte count, 2,400, with segmented neutrophils 46, band cells 44, lymphocytes 2 and monocytes 8 per cent. The neutrophils showed moderate toxic granulation. One normoblast was seen per hundred leukocytes. The blood platelets appeared to be moderately decreased in number. The bleeding time (Duke method) was five and a half minutes; the clotting time (capillary tube method), five minutes. The reticulocyte count was 5 per cent. The sickle cell trait was absent. There was a slight trace of albumin in the urine, and the urinary sediment contained occasional leukocytes, erythrocytes and finely granular casts. The benzidine test of the stool was weakly positive for occult blood. No ova or parasites were found. The Wassermann test of the blood was negative.

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The roentgenologist who examined the chest reported as follows: "The lung fields, especially the left, have hazy spotty densities, with very rounded infiltrations around the lung markings, about 1 to 2 mm. in diameter." This was interpreted as possible leukemic infiltration of the left lung.

Leukocyte concentration smears were made because of the marked leukopenia and anemia associated with hepatic and splenic enlargement. They revealed the organisms of *Histoplasma capsulatum* in occasional monocytes (fig. 1). Similar organisms were found without difficulty in smears made from the sternal marrow (fig. 2). These organisms were found in the peripheral blood smears made the night of admission but were not recognized at that time.

The patient's course in the hospital was progressively down hill. Anorexia was marked. Treatment consisted entirely of supportive measures. He received three blood transfusions, ferrous sulfate and supplementary vitamins, including vitamin K. During his stay in the hospital his temperature varied between 99 and 102 F. (37.2 to 39 C.). His pulse rate averaged 130 and the respiratory rate 36. The lesion over the right clavicle continued to bleed. Topical applications of a thromboplastic substance controlled the bleeding temporarily. Decubital ulcers appeared over the pressure points on his back. He died a few hours after the onset of delirium eleven days after admission to the hospital.

Necropsy (thirty-six hours after death).—The fat depots throughout the body were depleted. An ovoid granular red ulcer, measuring 10 by 14 mm., overlay the eroded surfaces of the right sternoclavicular joint. Three superficial decubital ulcers were seen on the back.

The skeletal muscles were well formed, pink and flabby. The axillary and inguinal lymph nodes were dark red and soft, with well defined cortical and medullary zones. They averaged 1 to 1.5 cm. in the greatest dimension.

There were 500 cc. of clear, bright yellow, watery fluid in the peritoneal cavity, 60 cc. of similar fluid in each of the pleural cavities and 100 cc. in the pericardial sac. The linings of these spaces were smooth, translucent and glistening.

The pale, flabby but well formed heart weighed 135 Gm. It contained soft sedimented clots.

The soft bilobed thymus which weighed 5 Gm. had a finely granular, pink cut surface.

The voluminous lungs together weighed 860 Gm. The pleurae contained numerous punctate hemorrhages. The anterior third of the pulmonary tissue was pink, reticulated and crepitant. The remainder of the lung was dusky purple, boggy and nonaerated. Copious, bright red, frothy fluid exuded from the cut surfaces, which were gray-red, spongy and marked with many slightly raised darker irregular areas 3 to 7 mm. in diameter. These lesions, more numerous near the hilus, were poorly delimited and seemed to be peribronchial in distribution. Some of them had tiny central yellow areas. The trachea and the bronchi contained frothy fluid. Their mucosae were yellow-red. The tracheobronchial lymph nodes varied from 2 to 4.5 cm. in the greatest dimension. They had red-gray, finely granular cut surfaces.

The large, pale liver weighed 760 Gm. A few gray subcapsular opaque areas, 2 mm. in diameter, were seen in the right lobe. Yellow material present in the periphery of each lobule accentuated the normal markings throughout the organ. A solitary, soft, bright yellow, structureless area 4 mm. wide surrounded by a thin fibrous capsule lay in the right lobe. The extrahepatic biliary system was normal.

The enormous, purple, firm spleen, which weighed 760 Gm., had a dark red homogeneous cut surface from which the pulp could be scraped easily.

No changes were recognized in the pancreas.

The esophagus, the stomach, the duodenum and the proximal half of the jejunum did not appear remarkable. In the distal half of the jejunum and in all of the ileum, on the free edges of the semicircular plicae were discrete firm gray nodules from 2 to 3 mm. in diameter. Similar nodules studded the mucosa of the colon. They were most numerous in the ileum, the cecum and the ascending colon and became gradually less plentiful as one proceeded in either direction from these regions. Some of the masses were surrounded by hyperemic halos, some appeared to be superficially ulcerated, and on cut section all were gray and structureless, although a few of the largest had small yellow cores. These lesions did not correspond to the distribution of intestinal lymphoid follicles, which were readily recognized.

The largest lymph nodes in the body lay in the mesentery and about the head of the pancreas. Many of them attained a diameter of 4 cm. and were gray-red and granular. The largest nodes had yellow, dry, structureless centers suggestive of caseous necrosis.

The adrenal bodies, which together weighed 15 Gm., were yellow-gray, marked with several bright yellow, sharply circumscribed, structureless lesions 1 to 2 mm. in diameter, confined to the cortical tissue.

Each of the pale kidneys weighed 90 Gm. Their capsules stripped readily, leaving smooth yellow-red cortical surfaces mottled with punctate hemorrhages near the lower poles. Similar hemorrhages were seen on the cut surfaces, in which cortical and medullary zones were well defined but the columns of Bertini were obscured. A dry yellow area of necrosis replaced the right lower renal papilla. The renal pelves and ureters appeared normal. The mucosal surface of the urinary bladder was studded with minute hemorrhages.

The external and internal genitalia, prepubertal in type, showed no changes of pathologic significance.

The leptomeninges were opaque and slightly thickened over the convexity of the cerebrum. Multiple sections of the well formed brain failed to reveal any structural changes. The pituitary body appeared normal.

A soft yellow area 1.7 cm. in diameter, with depressed center and scalloped edges, lay in the left frontal bone, extending from the galea aponeurotica to the dura mater. A similar area 8 mm. in diameter was found in the body of the tenth thoracic vertebra. It was surrounded by a thin zone of pale gray tissue. Elsewhere the vertebral marrow was red-gray and the femoral marrow, red-brown and gelatinous.

Microscopic Examination.—Organisms of two morphologically separate and distinct types, belonging to the fungi imperfecti, were found in the tissues of this patient. Those of the first type, which were more numerous, were basophilic, gram-positive spherical bodies 1 to 5 microns in diameter surrounded by thin achromatic halos. The central chromatin contained a small vacuole. No evidence of budding was found. These organisms were invariably intracellular but excited little inflammatory reaction. They conformed in their structural details to *H. capsulatum*.

The second type was doubly contoured, round or elliptic, gram-positive, from 5 to 25 microns in diameter, with little chromatin, and was surrounded by a thick, highly refractile capsule. Budding forms were present. These organisms were extracellular except for those that lay within foreign body giant cells. A peculiar

mucoïd substance was present when the yeast cells were found in large numbers. The organisms were most numerous in the leptomeninges. They conformed in their structural detail to *Cryptococcus neoformans* (*Torula histolytica*).

In the heart no changes of pathologic import were found.

In the lungs, many of the small pulmonary veins contained small mural thrombi, and some were completely occluded. Macrophages filled with histoplasmas were conspicuous in the thrombi and in the necrotic walls of the affected vessels (fig. 3). Lymphocytes formed a cuff about the necrotic areas, and there were small hemorrhages in the surrounding tissue. In some sections the venous lesions were so numerous that the hemorrhages coalesced. Tiny foci of pneumonia with fibrin and a few polymorphonuclear leukocytes were seen near some of the hemorrhages, but neither pneumonia nor parenchymal necrosis was a conspicuous feature of the pulmonary change. Relatively uninvolved portions of the lung were edematous. No histoplasmas were seen in the bronchial epithelium and only a few were found in the cells lining the pulmonary alveoli. No cryptococci were found.

In the liver, the cytoplasm of practically every Kupffer cell was filled with histoplasmas (fig. 4). Smaller numbers of the organisms were seen in hepatic cells, but none were seen in the biliary epithelium. The isolated focus was a mass of coagulation necrosis and cryptococci surrounded by foreign body giant cells and enclosed in a thin capsule of dense collagenous tissue (fig. 5). Only a few histoplasma-laden macrophages were seen in this lesion. The hepatic structure was well retained. Parenchymal cells in the periphery of each lobule contained large intracytoplasmic sudanophilic vacuoles. The central zones were hyperemic, and some of the cells showed the early changes of necrosis.

The pulp of the spleen was intensely congested. Many of the splenic follicles were made up of reticulum cells with few lymphocytes. Large numbers of histoplasmas were found in the reticulum cells in the follicles and in those lining the sinusoids. No cryptococci were found.

Occasional foci of coagulation necrosis were found in parenchymal and interstitial tissue of the pancreas. The number of islets was reduced, and probably some of the necrotic foci represented damaged islets. Histoplasmas were seen within macrophages in the necrotic foci and in thrombosed veins. No cryptococci were found.

No pathologic changes were encountered in the stomach or the duodenum. There was advanced autolysis of the luminal cells throughout the intestines. Histoplasma-laden macrophages formed nodules confined to the tunica propria of the small bowel, particularly at the tips of villi. Some of the nodules had small central necrotic areas. Acute inflammatory reaction was absent. The lymphoid follicles were not involved. Similar lesions were seen in the mucosa and the submucosa of the colon.

In the adrenal glands cortical lipid was depleted. Two types of lesions were seen in the cortices. The smaller, generally 50 to 100 microns in diameter, were composed of groups of necrotic cells, histoplasma-laden macrophages and polymorphonuclear leukocytes. The larger lesions, visible macroscopically, were sharply outlined groups of swollen cells, probably macrophages, filled with histoplasmas. A few histoplasmas were seen also in adrenal cortical cells that appeared structurally normal. Small veins in the cortex and the medulla contained thrombi in which histoplasmas were found. No cryptococci were seen.

In the kidneys, small focal necroses containing histoplasma-laden macrophages distorted the cortical structure. Foreign body giant cells were seen about some of these foci. The lesions appeared to be vascular in origin, since renal venules were

thrombosed and one area of necrosis had the configuration of a small infarct. An encapsulated focus of cryptococci in the lower papilla of the right kidney resembled the lesion found in the liver. Histoplasmas were seen in structurally normal glomeruli, in epithelium lining proximal convoluted tubules and in normal endothelium lining veins in the absence of thrombosis.

The submucosa of the bladder contained focal accumulations of histoplasma-laden macrophages.

No pathologic changes were seen in the prostate.

In the testes, there were focal collections of histoplasma-laden macrophages in the tunica albuginea.

In the lymph nodes, the earliest change, seen best in the axillary and tracheo-bronchial lymph nodes, was a marked hyperplasia of sinusoidal endothelial cells, which were filled with histoplasmas. Lymphoid follicles were scarce and germinal centers absent. Small foci of necrosis were seen among the parasitized cells. The intra-abdominal nodes contained numerous focal necroses and large caseous areas. All of the organisms seen were typical histoplasmas except those in one node, where a large encapsulated area of cryptococci and foreign body giant cells embedded in a mucinous matrix was found.

No organisms were seen in the involuted thymus.

The leptomeninges were edematous, containing macrophages, few lymphocytes, polymorphonuclear leukocytes and focal areas of foreign body giant cells within which typical cryptococci were seen. Extracellular cryptococci lay in the meninges and filled some of the Virchow-Robin spaces (fig. 6). There were no parenchymal lesions. Histoplasma could not be identified with certainty in either the leptomeninges or the brain tissue.

The femoral marrow contained few granulopoietic cells, rare adult granulocytes and little fat. Normoblasts and earlier erythropoietic forms were numerous. The predominating cells were large and ovoid, with eccentrically placed nuclei and pale acidophilic cytoplasm filled with histoplasmas. They were thought to be a mixture of reticulum cells and macrophages. Only scattered small foci of hemopoiesis were present in the vertebral marrow, which was largely replaced by histoplasma-laden cells like those in the femoral marrow. The region of the abscess in the tenth thoracic vertebra contained large numbers of cryptococci mixed with

EXPLANATION OF FIGURES 1 TO 12

Fig. 1.—Yeast form of *H. capsulatum* in the cytoplasm of a monocyte in a concentration smear of peripheral blood; Wright's stain; $\times 1,055$.

Fig. 2.—Yeast form of *H. capsulatum* in the cytoplasm of a monocyte from a smear of sternal marrow; Wright's stain; $\times 1,055$.

Fig. 3.—Small pulmonary blood vessel showing a mural thrombus containing histoplasmas; hematoxylin and eosin; $\times 234.5$.

Fig. 4.—Histoplasmas in Kupffer cells of the liver; hematoxylin and eosin; $\times 234.5$.

Fig. 5.—Cryptococci in a necrotic focus of the liver; hematoxylin and eosin; $\times 234.5$.

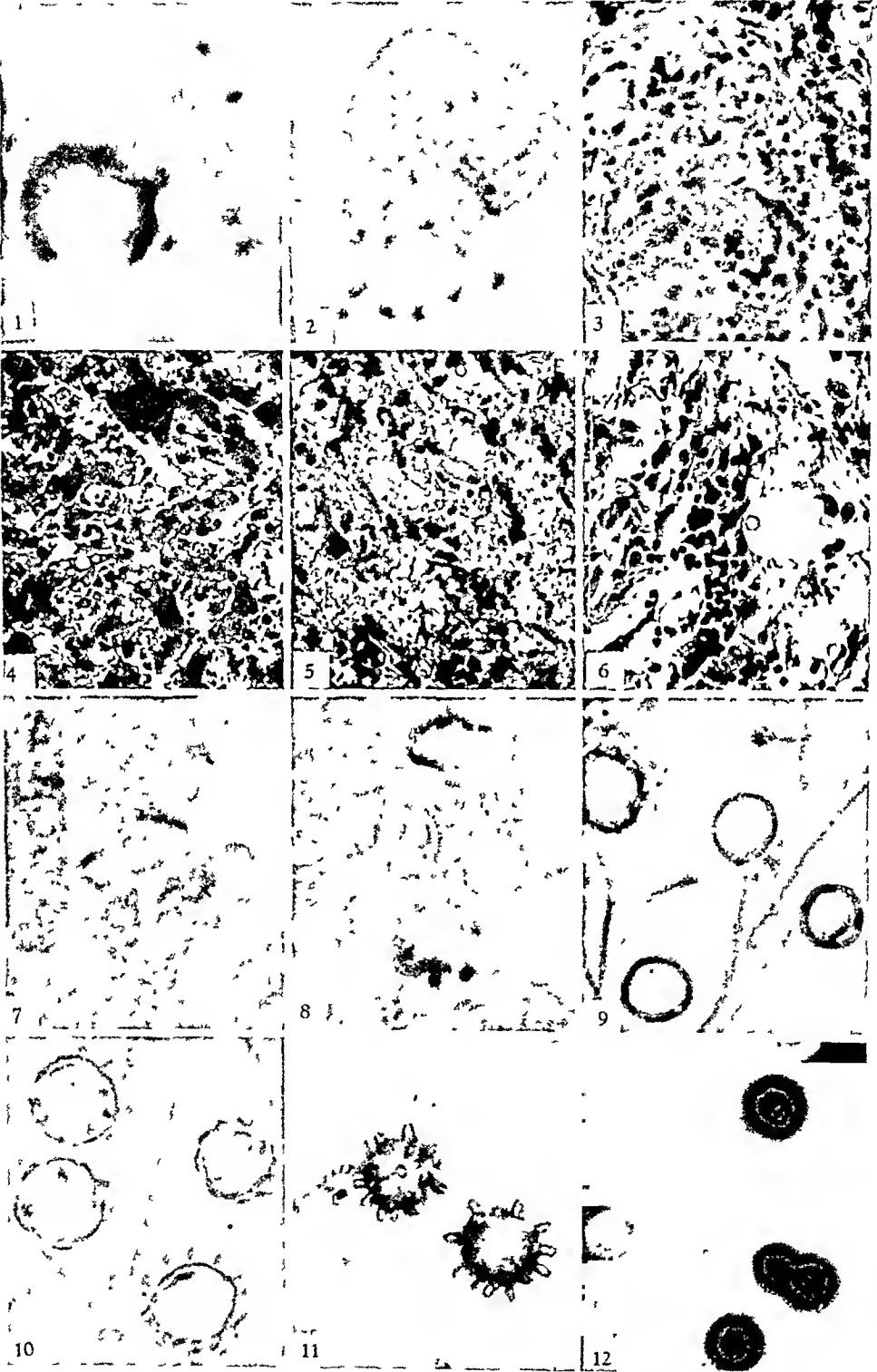
Fig. 6.—Cryptococci in the leptomeninges; hematoxylin and eosin; $\times 234.5$.

Figs. 7 and 8.—Cryptococci in pus, showing capsules; Greenhal's stain of a wet preparation; $\times 539$.

Figs. 9 and 10.—*H. capsulatum* from a slide culture, showing different stages in the development of tuberculate chlamydospores; $\times 539$.

Fig. 11.—*H. capsulatum* from a slide culture, showing characteristic brown tuberculate chlamydospores; $\times 539$.

Fig. 12.—*C. neoformans* from a three day old growth on Sabouraud's agar; india ink preparation showing capsules; $\times 539$.



(See legends on opposite page)

a few histoplasmas. Many of the bony trabeculae had a mosaic pattern due to the presence of hyaline cartilage. The right sternoclavicular joint space was filled with cryptococci and a few intraacellular histoplasmas. Two thirds of the articular surface of the clavicle was eroded. Marrow spaces in the epiphysial remnant were fibrosed. Short, blunt mosaics of bone and cartilage lay in the region of endochondral ossification. Histoplasmas and cryptococci were found throughout the clavicular marrow, but the latter were particularly numerous in the metaphysial portion.

Mycologic Studies.—With a pipet, material was aspirated from the draining lesion in the region of the right clavicle. This serosanguinous exudate contained clumps of pus cells among which were scattered single and budding yeast cells. Greenthal-stained wet preparations showed that the largest yeast cells exceeded the leukocytes in size and had sufficiently wide capsules to suggest *C. neoformans* (*T. histolytica*) (figs. 7 and 8).

Wright-stained smears of the pus showed many single and budding yeast cells which varied greatly in size. The smaller ones were frequently found within the cytoplasm of monocytes and occasionally in neutrophils. They stained blue and were usually 2 to 4 microns in diameter, but occasionally their size equaled that of the red blood cell. Except for the marked variation in size, the intracellular organisms closely resembled the histoplasmas seen in the blood smears. It was noted, however, that some of the nuclei were round with achromatic centers instead of having the usual crescentic shape. The extracellular organisms varied in size from those previously described up to large single budding forms 10 to 12 microns wide and 15 to 18 microns long, including the capsules. The width of the pink-stained capsule was about one-fourth the diameter of the blue-stained yeast cell but became progressively less distinct as the organisms became smaller. In the larger yeast forms the pink area surrounding the cell quickly faded peripherally into a relatively broad achromatic zone, the outer border of which was outlined by the surrounding cells or sometimes by a line of finely precipitated stain.

In culturing the blood the centrifuged sediment of oxalated blood remaining after a portion of the leukocyte-containing layer had been removed for concentration smears was poured into two blood agar plates which were sealed and then incubated at 37 C. A week later several minute yeastlike colonies which had appeared were transplanted to Sabouraud's agar test tube slants, and the plates were left at room temperature. Fuzzy white mycelial colonies were first seen on one of the blood agar plates ten days after inoculation. They were transplanted to three van Tieghem's cultural cell slides prepared with Sabouraud's agar. The first culture to show tuberculate chlamydospores characteristic of *H. capsulatum* was a yeastlike transplant on Sabouraud's agar after one month's growth (figs. 9 and 10). A van Tieghem's cell transplant of this culture made four days before the development of definite tuberculations on the chlamydospores, however, was characteristic of *H. capsulatum* within nine days. The variability in the time required for the development of tuberculate chlamydospores is further illustrated by the contrasting observation that two slide cultures made by direct inoculation from three week old blood agar plate colonies required six weeks for maturation. The tuberculate chlamydospores became brown in about one month (fig. 11).

Organisms of one of the yeastlike colonies early transplanted from the blood agar remained in the yeast form at room temperature both in old (two and one-half month) cultures and in a series of frequently transplanted colonies. India ink preparations were then made of one of the latter, and among many single-budding yeast cells were seen a few large encapsulated forms typical of *C. neoformans*.

Exudate from the draining area in the right clavicular region was inoculated on blood agar, and yeastlike organisms were isolated from this culture. Numerous transplants were made, including slide cultures. With two exceptions the organisms remained in the yeast form at room temperature, both in old (five month) and in frequently transplanted cultures. The yeast was equally luxuriant in the incubator (37 C.) and at room temperature. The growth initially was white or cream colored and turned to light brown in a few months. India ink preparations of a five day old, fourth generation blood agar transplant showed that the larger yeast-like cells had well developed capsules characteristic of *C. neoformans* (fig. 12). The capsules of these organisms are frequently smaller when examined in cultures than when examined in pus or spinal fluid.

Two van Tieghem's cell Sabouraud agar slides, A and B, were inoculated from an original blood agar culture. Mycelial growth appeared after one week in A. Many terminal chlamydospores were present within two weeks. These, however, did not show definite tuberculations characteristic of *Histoplasma* until two months later. Two transplants made in the interim from this slide culture grew out as encapsulated yeast cells. Hence, it is inferred that this was a mixed culture of *Histoplasma* and *Cryptococcus*. (See animal inoculations.) Slide culture B showed mycelial growth with the development of smooth-walled chlamydospores which never developed to the tuberculate stage.

Examination of a two month old slide culture transplant from A showed that some of the larger thick-walled yeast cells had given rise to rudimentary coarse filaments. Similar observations have been made on *Cryptococcus* cultures by Weidman¹ and Conant.²

Animal Inoculations.—The first inoculations carried out were with the yeast form of the fungus obtained by culture of pus. One cubic centimeter of a saline suspension made from the surface growth on a fourth generation Sabouraud's agar slant was injected intraperitoneally into each of 2 mice. One of the mice died twelve days after inoculation, and the other was obviously ill when killed and examined two days later. Cultures were made of material from the spleen and the liver on Sabouraud's agar. A luxuriant yeast growth was obtained in each case, and india ink preparations of the splenic culture contained, among many smaller single-budding yeast cells, a few large forms with well developed capsules characteristic of *C. neoformans* (*T. histolytica*).

Other inoculations of mice were carried out as follows: 1. The inoculum consisted of yeast recovered from a Sabouraud's agar transplant from slide culture A. The mouse was moribund on the seventeenth day, when it was killed and an autopsy made. Blood agar cultures of spleen showed single-budding yeast cells four days later. Several of the cells had large capsules. 2. The inoculum was a Sabouraud's agar culture of yeast isolated from the patient's blood. This mouse died seventeen days after inoculation, and cultures were made at autopsy. India ink preparations again indicated *C. neoformans*, and a majority of the yeast cells showed well developed capsules in a culture five months old. 3. A saline suspension prepared from a fifth generation Sabouraud's agar transplant of *H. capsulatum* from a blood culture was used as the inoculum. The mouse never showed any signs of

1. Weidman, F. D.: Pathogenic Yeasts, Molds and Actinomycetes, in Park, W. H., and Williams, A. W.: Pathogenic Micro-Organisms, ed. 11, Philadelphia, Lea & Febiger, 1939.

2. Conant, N. F., and others: Manual of Clinical Mycology, Philadelphia, W. B. Saunders Company, 1944.

illness, and at autopsy, six weeks later, no macroscopic or microscopic lesions were found. Cultures of the omentum, however, were identical with previous cultures of *Histoplasma*.

The histologic changes observed in the mice that were inoculated intraperitoneally with cultures resembling *C. neoformans* were similar. Brain, liver, spleen, lungs, kidneys, omentum, intra-abdominal lymph nodes and retroperitoneal fat contained cystlike spaces as much as 3 mm. wide filled with encapsulated yeast cells varying from 3 to 25 microns in diameter. The yeast cells had round nuclei. They were always extracellular, and despite the large size that some of the lesions attained, inflammatory reaction was minimal. Numerous organisms were found in the leptomeninges. The distribution and the appearance of the lesions conform to those described by Kessel and Holtzworth³ as characteristic of the reaction to *Cryptococcus*. The results of our inoculations of *H. capsulatum*, as already indicated, agree with those of Parsons,⁴ who has shown that intraperitoneal injection of *H. capsulatum* produces no lesions in mice. Intravenous inoculations produce systemic disease, but these were not attempted.

COMMENT

The differences in the structure and the cultural characteristics of the two yeasts isolated from this patient and in the lesions produced both in the patient and in mice by them indicate clearly that a mixed infection existed. Apparently the intestinal tract served as the portal of entry for the *Histoplasma*, since the oldest lesions were found in the ileum and the mesenteric lymph nodes. When parasitized macrophages were disseminated by way of blood and lymph channels, widespread changes were produced, among which venous thrombosis was prominent. It is probable that the mechanism by which the thrombi were formed involves the engulfing of histoplasmas by endothelial cells with subsequent damage to the vascular lining and thrombus formation. The portal of entry for the cryptococci seems less clear, although a single intra-abdominal lymph node contained a large focus of these organisms. It may be inferred that the patient was infected simultaneously by both *H. capsulatum* and *C. neoformans*. The recognized predilection of cryptococci for the leptomeninges was well illustrated. Only a few small foci of cryptococci were found elsewhere, and most of these also contained histoplasmas.

Little is known of the natural reservoirs of either *Cryptococcus* or *Histoplasma*, but concomitant infection with two different fungi, a rarity even among dermatomycoses,⁵ suggests that they may have come from a common source.

SUMMARY

A Negro boy died approximately three months after the onset of a subacute febrile illness. *C. neoformans* (*T. histolytica*) and *H. capsulatum* were grown from the blood and from exudate. Lesions produced by each of these fungi were found at autopsy.

3. Kessel, J. F., and Holtzworth, F.: *Am. J. Trop. Med.* **15**:467, 1935.

4. Parsons, R. J.: *Arch. Path.* **34**:229, 1942.

5. Muskatblit, E.: *Arch. Dermat. & Syph.* **44**:631, 1941.

MYELOFIBROTIC ANEMIA IN HODGKIN'S DISEASE

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ANEMIA is so constant a finding in Hodgkin's disease, particularly in the terminal stages, that it can be considered an integral feature of the disease. In the usual case it is of "secondary" type, not particularly severe, and is vaguely attributed to nutritional, toxic or infectious factors or, less specifically, it is considered to be an inevitable accompaniment of the cachexia which characterizes the disease. Although involvement of the skeletal system is a common complication of Hodgkin's disease, it frequently represents an incidental postmortem observation, its incidence increasing in direct proportion to the diligence of the examination. Less frequently, involvement of bone is an important feature of the clinical picture, producing pain, with destructive infiltration readily demonstrable by roentgenologic examination. It appears that severe anemia of myelofibrotic¹ type resulting from widespread involvement of the hemopoietic marrow is unusual in Hodgkin's disease.

REPORT OF A CASE

Miss I. H., a 60 year old woman, was admitted to the service of Dr. C. W. Morton at the Presbyterian Hospital on April 23, 1945 with a history of fever for a period of one month preceding admission. Her temperature rose to 100 or 101 F. daily. The remainder of the recorded history was irrelevant except for nocturia of many years' duration and occasional hematuria.

The patient was well developed and well nourished. No physical abnormalities were observed. The red blood cells numbered 3,500,000 per cubic millimeter; the hemoglobin content was 70 per cent; the white blood cells numbered 5,000 per cubic millimeter. The differential count was normal. The sedimentation rate was 18 mm. in thirteen minutes.

Throughout the one week period of hospitalization there was a low grade fever, with a maximum temperature of 101 F.

On July 9 the patient was readmitted complaining of fever and chills of two days' duration. Since leaving the hospital she had noticed occasional ecchymotic areas in the skin.

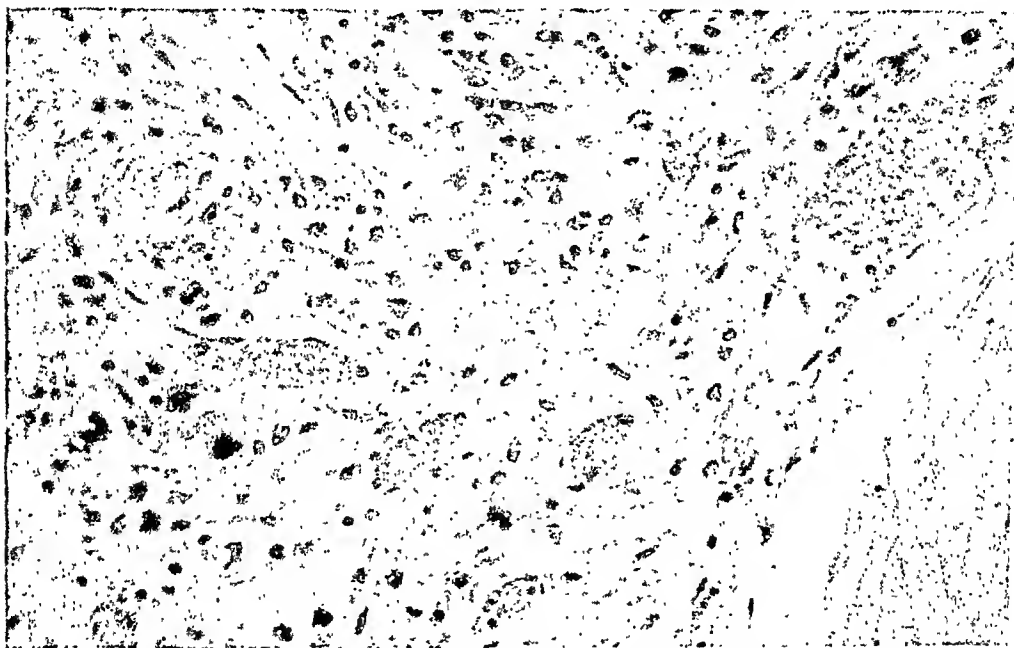
The physical findings were normal except for scattered ecchymoses. The red blood cell count varied between 3,500,000 and 4,000,000; the hemoglobin content was 70 to 80 per cent; the platelets numbered 239,000 per cubic millimeter; clotting time was three minutes and forty seconds, and bleeding time was three minutes. The red blood cells appeared normal in size and hemoglobin content. Blood cultures and agglutination tests for typhoid, paratyphoid and undulant fever were negative. The sedimentation rate remained rapid. The patient stayed in the hospital for one week, two days of which she was febrile, with maximum elevations of temperature of 101 and 102 F.

From the Department of Pathology, University of Pittsburgh, and the Presbyterian Hospital.

1. The term "myelophthitic" has been applied to this type of anemia.

The third admission was on September 5, because of severe urgency and frequency of urination. Retrograde pyelography revealed slight dilatation of the right renal pelvis. The urine from the right kidney contained many white cells, and culture yielded *Bacillus coli communior*. The red blood cell count varied between 2,500,000 and 3,400,000; the hemoglobin content, from 50 to 80 per cent. The results of additional laboratory tests were normal. Treatment during the three week period in the hospital consisted of ureteral catheterization of the right kidney and two transfusions of whole blood. During this period a remittent fever with peaks of 101 to 102 F. was recorded.

The fourth hospital admission was on October 4, for cystoscopic treatment. The physical findings remained normal. The red blood cell count was 3,500,000; the hemoglobin content was 68 per cent. She was discharged after three days. The highest recorded temperature in this period was 102.4 F.



Section of vertebral marrow showing diffuse granulomatous infiltration replacing the hemopoietic tissue. $\times 300$.

The fifth and final admission was on October 18, for lethargy, listlessness and excessive fatigue, which became progressively more severe until death on December 19, nine months after the first admission. The anemia failed to respond to repeated blood transfusions, injections of a liver extract with added thiamine hydrochloride, liver, marrow extract and amigen.

During this admission tenderness over the lower border of the liver was noted. At no time was the spleen palpable. A cardiac murmur was heard at the apex on several occasions. A week before death icterus was noted, and moist rales were present in the middle and lower lobes of the right lung. The temperature was persistently intermittent with daily elevations to 102 to 103 F.

The laboratory findings during the last admission were as follows: The urine contained a trace of albumin; the specific gravity varied from 1.010 to 1.020. There was a progressive decline in the red blood cell count from 3,400,000 to 1,970,000; the hemoglobin content dropped from 70 to 41 per cent. The erythrocytes showed

slight anisocytosis and poikilocytosis. The reticulocytes were 3.4 and 6 per cent; the white blood cell count varied from 1,000 to 4,000, with the percentage of mature polymorphonuclear leukocytes ranging from 58 to 70. Serum protein was 4.5 Gm. per hundred cubic centimeters, with albumin 3.9 Gm. and globulin, 0.6 Gm. Blood cultures were repeatedly negative.

Autopsy (twelve hours after death).—External examination revealed nothing noteworthy except moderate malnutrition and slight icteric discoloration of the skin and the scleras. The cervical, axillary and inguinal lymph nodes were not enlarged.

The lungs showed passive congestion. In the lower lobe of the left lung there were irregular areas of pneumonic consolidation containing miliary foci of suppuration.

The heart was grossly normal.

The spleen was moderately enlarged. It weighed 465 Gm. and measured 15 by 10 by 6 cm. On section the pulp was dark red and studded with pale, slightly elevated fibrous nodules from 1 to 4 mm. in diameter.

Section through the liver revealed numerous small irregular pale infiltrations and circumscribed miliary nodules. There were a few larger lesions up to 1 cm. in diameter.

The peripancreatic and paravertebral lymph nodes were slightly enlarged, the largest measuring 3 cm. in greatest diameter. They were not adherent to one another. On section the tissue was pale, glistening, solid and of uniform appearance throughout.

The marrow of the lumbar and dorsal vertebrae, the ilium and the ribs was mottled in appearance, presenting extensive pale, glistening, soft fibrous areas intermingled with smaller irregular deposits of red marrow. The cortex in all areas was intact, and there was no apparent destruction of the spongiosa.

Microscopic Examination.—The lungs contained confluent areas of bronchopneumonia; several of the bronchioles were distended with purulent exudate.

In the myocardium there were a few small sharply circumscribed embolic abscesses.

The spleen contained numerous granulomatous nodules composed of coarse interlacing deposits of collagen containing small numbers of inflammatory cells of various types and scattered mononuclear and giant cells of the Sternberg-Reed type. Present throughout the intervening splenic pulp were similar cells in greater number.

A similar infiltration was noted throughout the liver; at times it resulted in coarse nodulations which had replaced areas of hepatic parenchyma.

Several of the lymph nodes showed extensive alteration of structure. There were considerable fibrosis and moderately heavy diffuse infiltration of lymphocytes, plasma cells, monocytes, and scattered giant cells with abundant acidophilic cytoplasm and oval lobate vesicular nuclei, frequently multiple. In areas eosinophilic leukocytes were numerous. Areas of ischemic necrosis, so characteristic of the advanced lesions of Hodgkin's disease, were not observed grossly or microscopically in any of the lymph nodes.

Sections of vertebrae, ilium and ribs revealed extensive granulomatous infiltrations which had largely replaced the hemopoietic tissue. Only occasional irregular islands of uninvolved marrow were observed.

Various types of inflammatory cells and giant cells of the Sternberg-Reed type were present throughout a cellular fibroblastic matrix. The bony trabeculae were essentially normal in appearance; no destruction of bone was evident. There

were localized areas of ischemic necrosis of the granulomatous tissue traversed by unaltered lamellas of bone.

Sections from the esophagus and the adrenal gland revealed minimal infiltration of Hodgkin's type. Small embolic abscesses were seen in the kidneys.

COMMENT

The widespread involvement of the bone marrow resulting in extensive replacement of the hemopoietic tissue was undoubtedly responsible for the severe and progressive anemia, the most significant feature of the clinical picture. The infiltration was of such character that it did not cause destruction of bone and, therefore, was not productive of the deep-seated bone pain so typical of the osteolytic lesions of Hodgkin's disease.

Without a representative portion of the marrow for histologic study the diagnosis could not be made clinically in view of the vague clinical features and the nonspecific laboratory findings. Even at postmortem examination the exact nature of the disease was not apparent on gross inspection, because of the minimal enlargement of the lymph nodes.

The extent of the process in the marrow stands in striking contrast to the relatively minor involvement of the lymph nodes. One might question the orthodox interpretation that the lesion of Hodgkin's disease always commences in the lymph nodes. However, until cases of Hodgkin's disease with lesions confined to the marrow are described, the involvement of bone seen in this case must be considered secondary to a process primary in lymphoid tissue.

SUMMARY

An unusual case of Hodgkin's disease with extensive infiltration of the hemopoietic marrow resulted in severe and progressive anemia of the myelofibrotic type.

Notes and News

Appointments, etc.—The Surgeon General has announced the appointment of Col. Raymond O. Dart, M. C., as director of the Army Institute of Pathology, Washington, D. C., to succeed Col. James E. Ash, recently retired.

A. E. Margulis has been promoted to the professorship of bacteriology in the New York Post-Graduate Medical School and Hospital, made vacant by the death of Ward J. MacNeal.

Deaths.—Harry Plotz, 55, consultant to the Secretary of War after his retirement last year as head of the Virus Division, Army Medical Corps, died in Washington, D. C., January 6. He worked mainly with the typhus bacillus and mass typhus control, receiving the Legion of Merit in the recent war and foreign and United States recognitions in World War I.

Society News.—The newly organized American Academy of Oral Pathology will meet in the Stevens Hotel, Chicago, February 9. The secretary is Lieut. Col. J. L. Bernier, Army Institute of Pathology, Washington 25, D. C.

The American Association of Pathologists and Bacteriologists will hold its annual meeting Friday and Saturday, May 16 and 17, 1947, at the University of Illinois College of Medicine, Chicago, with headquarters at the Palmer House.

According to *Science*, the Union International Contre le Cancer has been invited by the directors of the American Association of Cancer Research to cooperate in holding the Fourth International Cancer Congress in St. Louis in September 1947. E. V. Cowdry, director of research in the Barnard Free Skin and Cancer Hospital and professor of anatomy at Washington University, has been appointed president of the congress. This invitation has been accepted by the Union International and Dr. Cowdry's appointment has been confirmed. Organization of the congress is well advanced and announcement of committees is expected soon.

Chorionepithelioma Registry.—The Albert Mathieu chorionepithelioma registry has been established by the American Association of Obstetricians, Gynecologists and Abdominal Surgeons. The purpose of the registry is to collect and study materials dealing with hydatidiform mole and choriocarcinoma. For the present the specimens will be stored in the laboratory of gynecologic pathology of the Johns Hopkins Hospital. The chairman of the committee in charge of the registry is Dr. Emil Novak, 25 East Preston Street, Baltimore. It is hoped that clinics and laboratories throughout the country will cooperate by sending material for study, classification and storage, for the purpose of advancing knowledge in a field of pathology in which there is now considerable confusion.

Counter Antivivisection Legislation.—The National Society of Medical Research, organized under the auspices of the Association of American Medical Colleges, is taking the lead in counter antivivisection legislation programs. Members and endorsers of the society include the Chamber of Commerce of the United States, the American National Red Cross and many other scientific groups. A. J. Carlson is president. The offices of the society are at 25 East Washington Street, Chicago.

Books Received

PRACTICAL HANDBOOK OF THE PATHOLOGY OF THE SKIN: AN INTRODUCTION TO THE HISTOLOGY, PATHOLOGY, BACTERIOLOGY AND MYCOLOGY OF THE SKIN WITH SPECIAL REFERENCE TO TECHNIQUE. By J. M. H. MacLeod, M.D., F.R.C.P. (Lond.), physician and honorary director of the department of pathology of St. John's Hospital for Diseases of the Skin; physician for skin diseases to the Hospital for Tropical Diseases, London; and I. Muende, M.R.C.P. (Lond.), M.B., B.S., B.Sc. (Lond.); physician with charge of the department of pathology and lecturer at St. John's Hospital for Diseases of the Skin, London. Third edition. Pp. 415, with 27 colored and 125 black and white illustrations. Price, \$10.50. New York: Paul B. Hoeber, Inc., 1946.

This book is recommended as a first class introduction to laboratory dermatology. Two pages, it is estimated, are devoted to gross anatomy, 4 to embryology, 74 to normal histology and 4 to the removing of specimens for biopsy. The pathology of the skin and appendages is discussed in 192 pages. Various pathogenic microorganisms which affect the skin are considered from the cultural and the microscopic point of view; animal parasites are discussed in 9 pages. Laboratory technic is presented in 90 pages. Much of the laboratory technic presented is rather old fashioned, although such a modern discovery as the dopa reaction is discussed and illustrated. The excellent modern staining methods of Masson are not mentioned.

Several statements of doubtful value are seen throughout the book. Hydradenitis suppurativa is of tuberculous origin. Corns may assume cancerous changes. Seborrheic warts exhibit marked acanthosis (as a matter of fact, no prickles can be demonstrated in the tumors). Hemosiderosis may be caused by exposure to heat.

The illustrations include chiefly microscopic drawings, which when as superb as are those used in the text are preferred to photomicrographs. Those presented in color are especially effective. The format of the book is excellent; the paper is of a good grade.

ALLERGY IN THEORY AND PRACTICE. By Robert A. Cooke, M.D., Sc.D., attending physician and director of the department of allergy, Roosevelt Hospital, New York; in association with Horace S. Baldwin, Robert Chobot, R. Clark Grove, Joseph Harkavy, Selian Hebdal, Michael Heidelberger, Paul Klemperer, Louis Schwartz, W. C. Spain, Dudley D. Stetson, Albert Vander Veer, Mathew Walzer and Margaret B. Strauss. Pp. 572, with 43 illustrations. Price \$8. Philadelphia and London: W. B. Saunders Company, 1947.

This book is the outcome of courses in cooperative postgraduate teaching of allergy by the author and associates. There are 9 sections with, in all, 32 chapters and an appendix: fundamental aspects of allergy (Cooke, Klemperer, Heidelberger); allergy of the bronchi, i. e., asthma (Spain, Cooke, Baldwin, Grove); allergy of the upper respiratory tract (Vander Veer, Hebdal, Spain); allergy of the skin (Cooke, Schwartz, Stetson); allergy of the nervous system (Cooke, Harkavy); allergy of the cardiovascular system (Harkavy); allergy in relation to specialties (Cooke, Chobot); allergens in relation to diseases of allergy (Cooke, Chobot, Baldwin, Harkavy); technics (Walzer, Margaret B. Strauss and Spain). In an appendix Cooke discusses nonspecific causes or influences in relation to allergic disease. There is a satisfactory subject index. At the end of each chapter is a list of the writings referred to in the text, complete in bibliographic detail. The foreword deals with medical education as related to allergy, in an effort to promote improvements in the present inadequate training facilities. The book will be useful to all interested in allergy—physicians, medical students, pathologists immunologists, bacteriologists, and scientists "who find in allergy an alluring subject on account of the intricacies of its immunologic phases." The aim of this book "to record the facts of allergy and, further, to synthesize them into a body of knowledge . . ., realizing fully the existing limitations of information and possible errors of interpretation," has been well realized.

DEVELOPMENT OF BONE MARROW IN ADULT ANIMALS

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AND

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THE DEVELOPMENT of cellular elements of bone marrow has been studied in fetal life, in young animals¹ and in cultures of explanted marrow.² Investigators have observed the progressive regeneration of marrow cells that were recovering from hypoplasia induced by chemicals,³ starvation⁴ and disease.⁵ In this article we present studies of the regeneration of the complete marrow, including stroma, fat spaces, frequently referred to as fat cells, and myeloid cells. The marrow was removed mechanically. Thus the generalized systemic effects incident to previously used methods were avoided.⁶

Bone marrow consists of stromal argyrophil reticulum fibers, reticular cells, fat spaces, blood vessels and myeloid elements composed of erythrocytes, granulocytes and megakaryocytes. Some investigators include monocytes. The role of the primitive reticular cell is involved in the subject of the well known controversy that has arisen among hematologists. The monophyletic school (Ferrata, Pappenheim, Maximow) believes that one primitive cell, variously labeled as hemocytoblast, lymphoidocyte and free-undifferentiated reticulum cell, gives rise to all types of blood cells. The polyphyletic school, including the duolists and trialists (Ehrlich, Nägeli, Piney, Sabin) ascribes multiple origins to blood cells. The composite and the more prevailing views of that school are that the myeloblast in the marrow gives rise to granular leukocytes, that the erythrocytes are derived from the endothelium of the vessels of the marrow and that the lymphocytes arise from the lymphoblasts of lymphoid tissue. The views on the origin of monocytes also vary;

From the Toledo Hospital Institute of Medical Research.

1. Sabin, F. R.; Metler, F. R.; Smithburn, K. D.; Thomas, R. M., and Hummel, L. E.: *J. Exper. Med.* **64**:97, 1936.

2. Osgood, E. E.: *J. A. M. A.* **109**:933, 1937. Rachmilewitz, M., and Rosin, D.: *Am. J. M. Sc.* **206**:17, 1943.

3. Muller, G. L.: *J. Exper. Med.* **43**:533, 1926.

4. Doan, C. A.; Cunningham, R. S., and Sabin, F. R.: *Contrib. Embryol.* **16**:163, 1925.

5. Peabody, F. W.: *Am. J. Path.* **2**:487, 1926.

6. Steinberg, B., and Martin, R. A.: *Proc. Soc. Exper. Biol. & Med.* **61**:428, 1946.

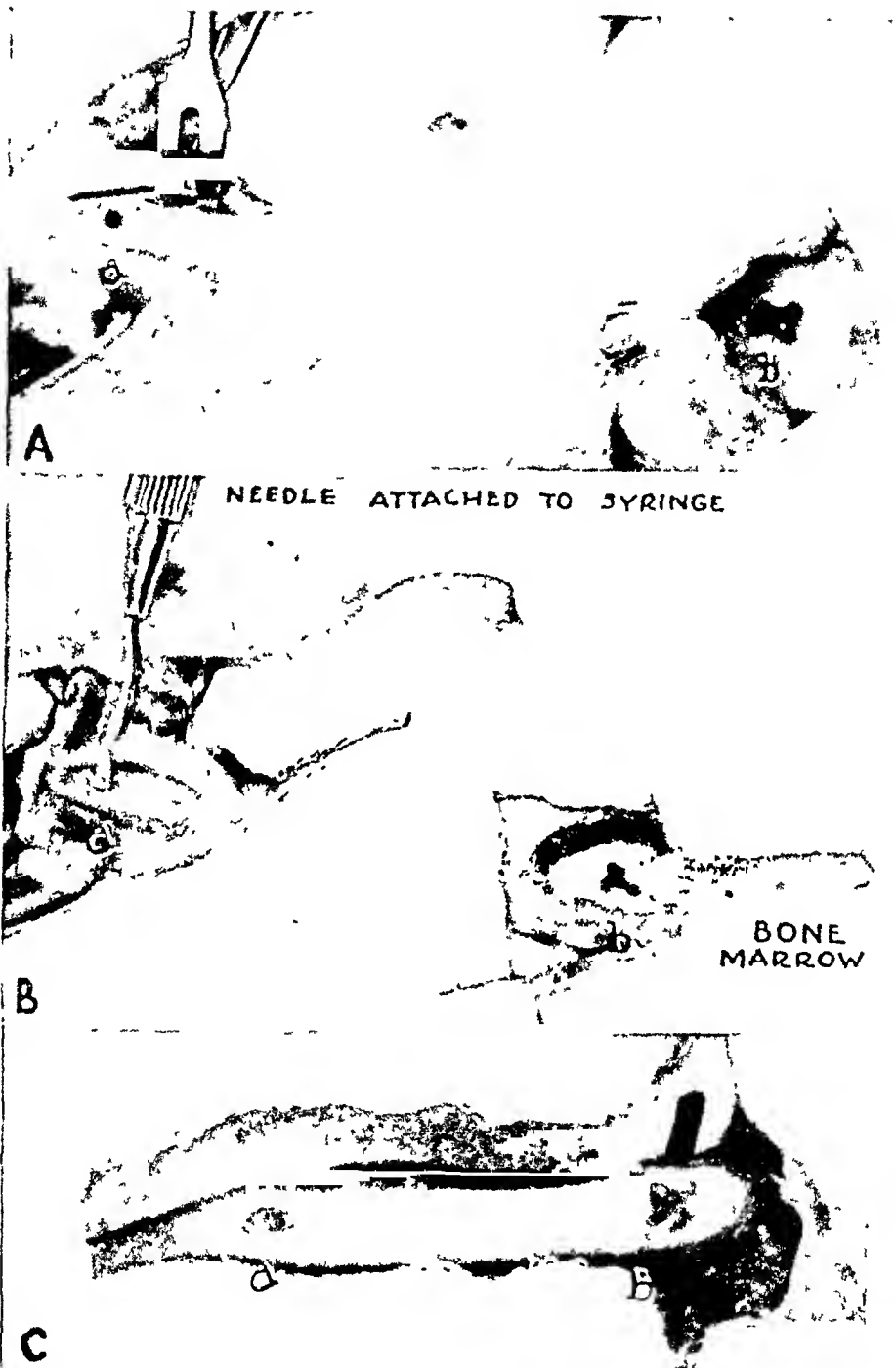


Fig. 1.—Removal of marrow from a long bone of a living rabbit. Holes are drilled in the bone with a Ralk nail drill. At one end of the bone a single hole is made (*a* in *A*). At the opposite end, four holes are made, and the central bone spicule is removed, leaving a large opening (*b* in *A*). A flexible silver cannula is inserted into the single hole (*a* in *B*), and with a syringe containing oil or water the marrow is expressed through the larger opening (*b* in *B*). Both openings are then sealed with bone wax (*a* and *b* in *C*).

the fixed macrophage, the vascular endothelial cell and the lymphoblast are the variously assumed sites of origin. There are many variables of these major theories of hemopoiesis.

The nomenclature of immature cells reflects in part the conflicting views of origin and development. Since the morphologic aspect of a developing cell is necessarily variable, a confusing motley of terms has come into being. For the purpose of this paper the following terms will be employed: "primitive reticular cell," which includes the intermediate forms; occasionally the term "hemocytoblast," used in the sense of an intermediate form of the primitive reticular cell; "myeloblasts"; "myelocytes"; "juveniles"; "stabs"; "mature polymorphonuclear

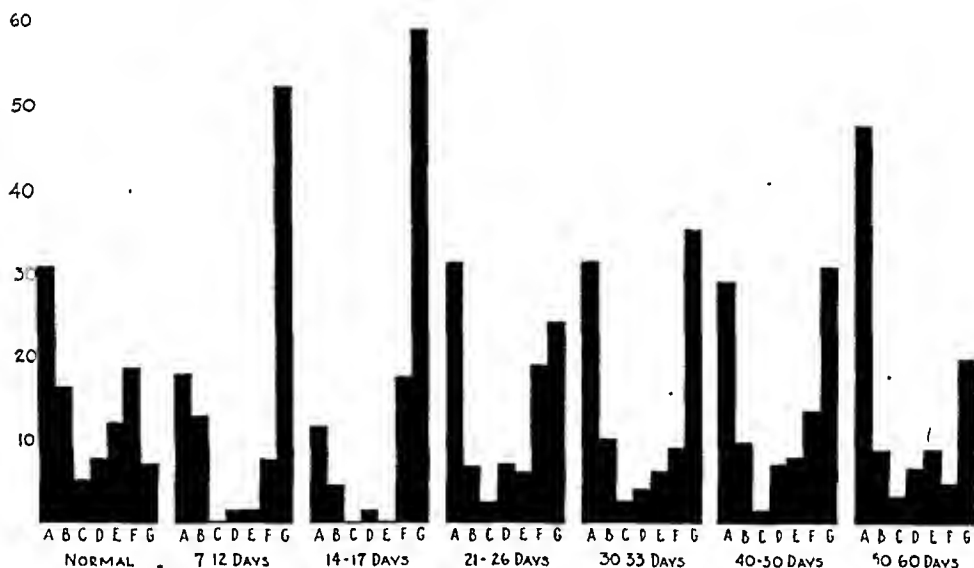


Fig. 2.—Distribution of myeloid cells of various forms in regenerating marrow at various periods following extirpation. *A*, nucleated red blood cells; *B*, polymorphonuclear cells; *C*, stabs; *D*, juveniles; *E*, myelocytes; *F*, myeloblasts; *G*, primitive cells.

cells." All the progenitors of the erythrocytes will be grouped as nucleated red blood cells, and the platelet precursors, as megakaryocytes and megakaryoblasts.

The primitive reticular cell is elongated and contains an ovoid nucleus with a fairly distinct perinuclear membrane and little chromatin. There may be one or two nucleoli. The cytoplasm is faintly basophilic, homogeneous and frequently not apparent. There are intermediate forms with round nuclei and with a greater amount of basophilic cytoplasm. As the primitive reticular cell assumes a more or less round shape, the chromatin increases in amount, the nucleus becomes round and occasionally indented, the cytoplasm becomes more basophilic and shows vacuoles. This cell is probably the hemocytoblast of Ferrata and of Maximow, Nägeli's myeloblast, Pappenheim's lymphoidocyte and the

lymphoid hemoblast of Jordan. The primitive reticular cell is seldom transformed to the hemocytoblast in the marrow normally. Under normal conditions hemopoiesis is homoplastic. Mature cells are derived from the younger forms. In pathologic states and under certain experimental procedures there is heteroplastic hemopoiesis, in which myelo-

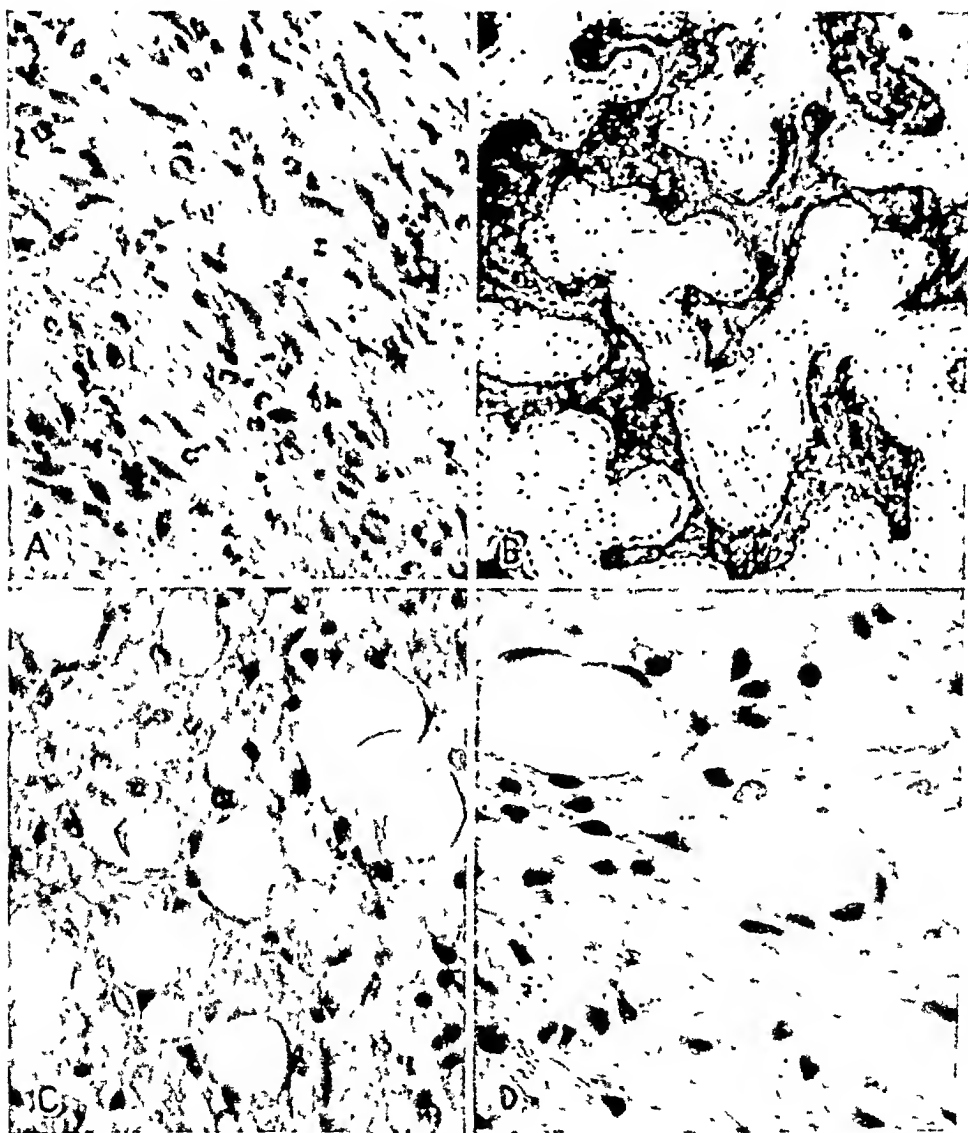


Fig. 3.—*A*, sheets of primitive reticular cells originating from the tibial endosteum nine days after extirpation of the marrow. Small capillaries are present with polymorphonuclear leukocytes that were derived from the circulation.

B, bone trabeculae extending from the tibial endosteum into the marrow cavity, with sheets of primitive reticular cells arising from the endosteum of the trabeculae.

C, grouping of two or more of the reticular cells. Vacuoles appear in the cytoplasm, and a few fat spaces begin to appear.

D, coalescence of the cytoplasm of reticular cells giving rise to fat spaces. The nuclei of reticular cells have migrated to the peripheries of fat spaces. Many reticular cells persist.

cytes and erythroblasts may be derived from the primitive reticular cell and its intermediate forms.

METHOD

The marrow of one or two of the long bones (tibias in these experiments) of the rabbit was extirpated by a method described previously.⁶ An incision was made at each end of a tibia. A single opening was made at the narrow end and four openings at the broad end with a Ralk nail drill. The piece of bone outlined by the four openings was lifted out. A tight fitting flexible silver cannula was inserted into the single opening. A syringe filled with sterile liquid petrolatum was attached to the cannula. The pressure of the oil separated the marrow and expressed it out of the bone cavity through the larger opening (fig. 1). The cavity of the bone was flushed out repeatedly with a warm solution of sodium chloride. In some of the animals the marrow was removed from the epiphyses as well as from the shaft. One or both of the ends were packed with inert material to prevent reformation of marrow.⁷ In other rabbits the epiphysial marrow was left intact.

Marrow was extirpated from 60 tibias (the right or both tibias) of 44 rabbits. The ages of the animals ranged from 8 to 10 months. One or more of the animals were killed at intervals of one to sixty days. The contents of the tibias were fixed in formaldehyde and Bouin's solutions. Sections were cut at various levels of the entire length and width of the marrow. The marrow was stained with hematoxylin, eosin and Giemsa preparations. The marrow was studied for the following factors: (1) rapidity and extent of formation of fat spaces and reticulum, (2) completeness with which the marrow refilled the tubular shaft of the tibia, (3) reestablishment of the normal proportions of the cell types and (4) the return to normal of the numerical content of individual and all cell types.

To determine the normal values, the absolute and the relative number of cells of each type were determined in the marrow removed at the onset of the experiment. Whenever only one tibial marrow was extirpated for regeneration, the cell content of the marrow of the other tibia was also determined when the animal was killed. As the animals were put to death, the cells of the regenerated marrow were counted and classified as to types.

RESULTS

Within the first nine days after the marrow was extirpated, the endosteum began to send out offshoots composed of sheets of primitive reticular cells and bone trabeculae (fig. 3 *A*). Extending from the endosteal layer of the trabeculae and bridging them were more sheets of primitive reticular cells (3 *B*). Some of the reticular cells began to change from ovoid to round forms, and vacuoles appeared in the cytoplasm, which became more abundant and basophilic. Two or more of the reticular cells became grouped, the cytoplasmic vacuoles coalesced and a fat space was formed (fig. 3 *C* and *D*). The formation of fat spaces continued for at least two months. Capillaries began to appear within the nine day period (fig. 3 *A*). Myeloid cells were few and

7. Steinberg, B., and Martin, R. A.: *Proc. Soc. Exper. Biol. & Med.* **63**:390, 1946.

scattered. Nucleated red blood cells predominated. Mature polymorphonuclear leukocytes were within capillaries and were derived from the circulation.

Between twelve and seventeen days after extirpation of the marrow, islands of hemocytoblasts, myeloblasts and erythroblasts and an infre-

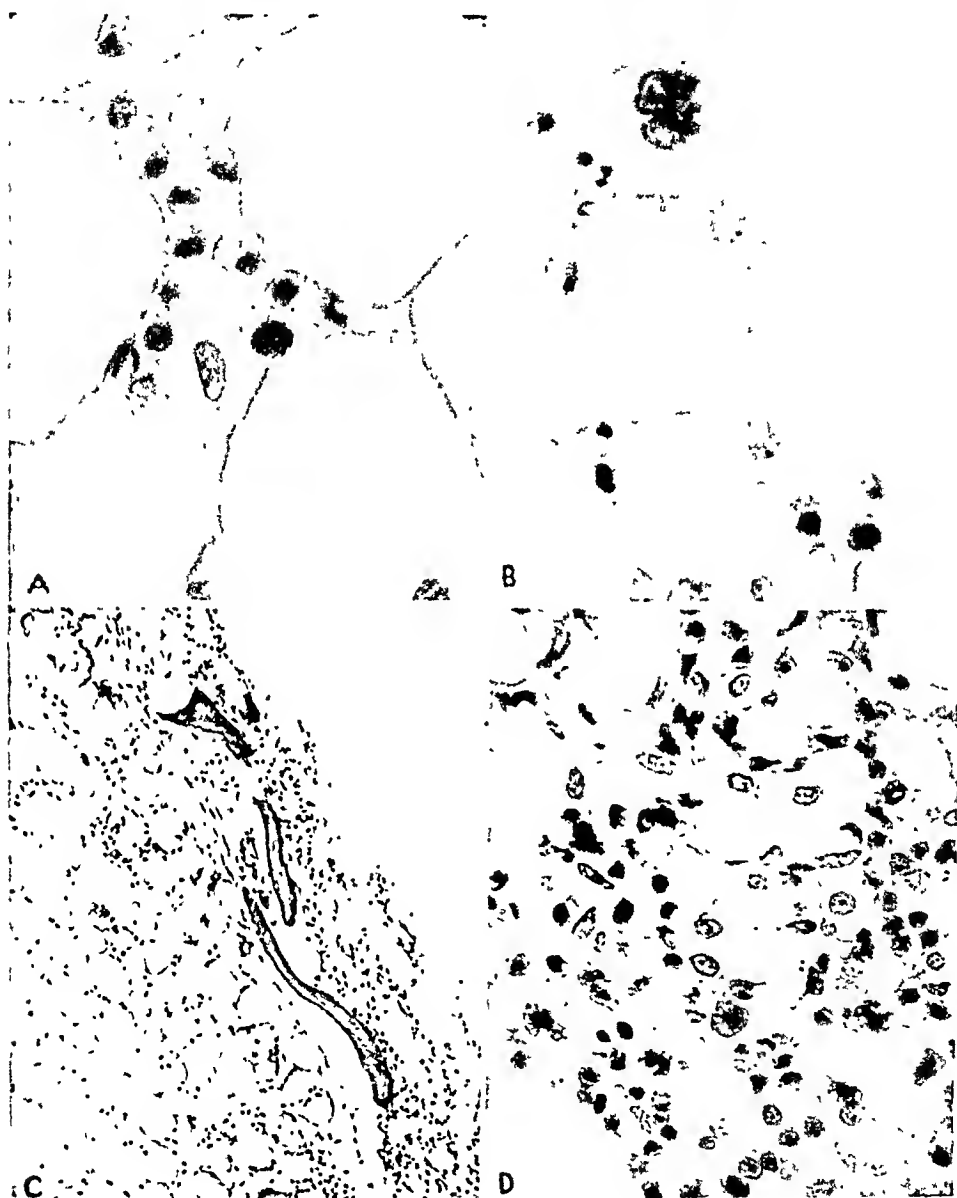


Fig. 4.—*A*, one of the islands of myeloid cells appearing from the twelfth to the seventeenth day after extirpation of marrow. In *B* there is a megakaryoblast.

C and *D*, regeneration twenty-one days after extirpation of marrow. The marrow cavity is almost completely filled, but the development of the marrow is not uniform. Bone trabeculae and small islands of reticular cells persist. The myeloid cell content is still small in numbers.

quent megakaryoblast were seen throughout the reforming marrow (fig. 4 *A* and *B*). The myeloid cells were found to congregate at the margins

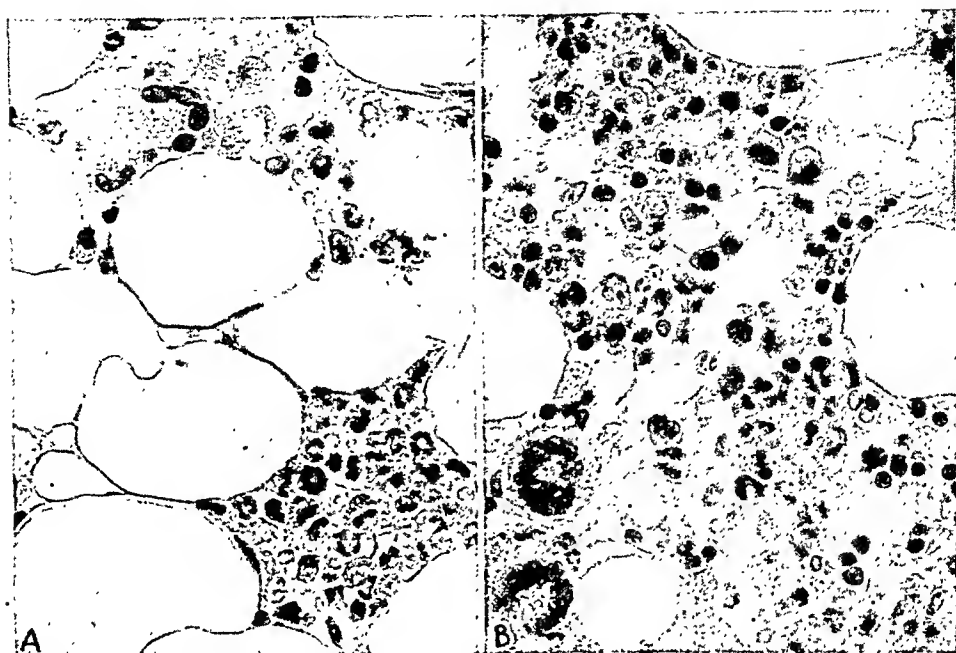


Fig. 5.—*A*, regeneration thirty days after extirpation of marrow. The myeloid areas are of average normal proportions. There is formation of a fat space at the upper left.

B, regeneration forty-three days after extirpation of marrow. Megakaryocytes appear in numbers consistent with normal marrow. There is a total increase in myeloid cells.



Fig. 6.—*A*, shaft of a bone from which marrow has been completely removed. The animal was killed to determine the efficacy of the procedure of complete removal of marrow. *B*, normal marrow which was removed from the shaft. *C*, regenerated marrow present thirty days after marrow had been completely removed from the shaft of a tibia.

of, or between, fat spaces. Primitive reticular cells predominated (fig. 2). The calcium of the bone trabeculae became reduced or disappeared entirely.

On the twenty-first day after extirpation the greater part of the marrow cavity had become filled. However, there was no uniformity of appearance. In some areas there was slight to moderate myeloid cellularity, with all the cell types represented. In other places the bone trabeculae and small collections of primitive reticular cells persisted (fig. 4 *C* and *D*). The relative formation of marrow elements suggested that development of fat spaces was prerequisite for formation of myeloid cells. Nucleated red blood cells and myeloblasts predominated (fig. 2).

The disappearance of bone trabeculae began with the absorption of calcium. The endosteal layer with its osteoblasts migrated away from the trabeculae and became a part of reticular cell sheets. The fate of the osteocytes was difficult to evaluate. Some of them showed gradual stages of disintegration and final absorption; others appeared to have become integrated in the myeloid development. The disappearance of bone trabeculae was by no means uniform. They persisted in some animals for sixty days after extirpation of marrow.

In thirty days the number of myeloid cells increased appreciably (fig. 2). Erythroblasts began to appear in larger numbers. Whereas in some areas the marrow was within the average normal in numbers and distribution of myeloid cell types, in other parts the picture was not unlike that of nine to seventeen days after extirpation (fig. 5 *A*).

Approximately after thirty days, megakaryocytes began to appear in numbers consistent with average normal distribution. The total number of myeloid cells became increased (fig. 5 *B*).

Within the first thirty days after extirpation the observations tended to indicate that homoplastic hemopoiesis represented the sole mechanism of cell development. In subsequent periods there were obvious difficulties in determining whether heteroplastic hemopoiesis took over or whether both mechanisms continued to operate.

From the fiftieth day the erythroblastic elements became hyperplastic. The other myeloid cell types were less in number than in the average normal marrow (fig. 2). In most instances the marrow had completely filled the cavity. The average normal quantity of cells was reestablished in the greater part of the marrow. There remained, however, infrequent islands of primitive reticular cells and partly formed fat spaces.

In the bones with one or both of the epiphysial ends sealed off to prevent reformation of marrow, regeneration in the shafts was decreased appreciably. The primitive reticular cells persisted in greater numbers for longer periods. The total myeloid content remained appreciably

lower even at the end of two months. Eventually the entire shaft became filled with marrow.

Comparison of the regenerating marrow of the adult rabbit with that of the fetus and the young animal suggests a comparable development (fig. 6).

SUMMARY

The complete marrow of one or both tibiae was extirpated in 44 living rabbits. The marrow was allowed to regenerate. Animals were



Fig. 7.—Bone marrow of a rabbit one day after birth. Note the bone trabeculae, the sheets of primitive reticular cells in the upper part of the photograph, the few fat spaces, and compare with figure 3 *B*, representing regenerating marrow as it appears in an adult animal nine days after extirpation of marrow. Note the similarity of the two marrows.

killed at intervals for a period of two months, and regeneration was studied. The probable development of marrow is indicated within the limits of a morphologic experiment.

The reformation of marrow began from the tibial endosteum with offshoots of primitive reticular cells and formation of bone spicules.

Approximation of two or more of the primitive reticular cells gave rise to fat spaces.

Presence of fat spaces was prerequisite to formation of myeloid elements in the marrow. Regeneration was not uniform. In various parts of marrow there was considerable variation in formation of fat spaces, reestablishment of normal proportions and numerical contents of cell types, and refilling of the tubular shaft of the bone.

Regeneration of marrow in adult rabbits apparently followed closely the pattern of fetal development.

Granulocytic leukocytes, erythrocytes and megakaryocytes were derived apparently from a single primitive reticular cell.

PATHOLOGIC ACTION OF DDT AND CERTAIN OF ITS ANALOGS AND DERIVATIVES

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SINCE the previous report¹ on the pathologic changes incident to experimental intoxication with 2,2-bis-(parachlorophenyl)-1,1,1-trichloroethane (DDT), we have studied the lesions in a small additional series of animals exposed to that compound and those in small series of animals, chiefly rabbits, exposed to various substitution products, derivatives and analogues of DDT as listed in the paper² dealing with the pharmacologic aspects of these compounds.

While the group of animals exposed to each compound was small, pathologic changes of interest were observed. Since some of these compounds may not be studied further at this time, it seems indicated to place on record the lesions observed with each one.

DDT

Autopsies were made on 10 rats fed DDT for a period of one year, this compound having been incorporated in their diets as follows: diet 200 (0.02 per cent DDT), 3 rats; diet 201 (0.05 per cent DDT), 3 rats; diet 202 (0.1 per cent DDT), 4 rats. Two additional rats fed DDT for two hundred and thirty days and three hundred and fifteen days died of suppurative and caseating monocytic pneumonia.

Most of the rats presented moderate to rather pronounced hypertrophy of the bronchial lymphadenoid tissue, some also focal perivascular lymphocytic infiltration of the parenchyma. Besides the 2 animals already noted, 2 more presented focal pneumonia of purulent type, and another, foci of epithelioid cell organization in alveoli. These pulmonary changes are to be regarded as infectious in nature, and the influence of the prolonged intoxication is uncertain.

From the Pathology Laboratory and the Division of Physiology, National Institute of Health, Bethesda, Md.

1. Lillie, R. D., and Smith, M. I.: Pub. Health Rep. 59:979, 1944.

2. Smith, M. I.; Bauer, H.; Stohlman, E. F., and Lillie, R. D.: Federation Proc. 5:203, 1946; J. Pharmacol. & Exper. Therap. 88:359, 1946.

The heart muscle presented a variable amount of stippling of the muscle fibers with very fine to fine fat droplets, in areas or diffusely, accompanied by more or less obscuration of the cross striae. In general this change was least with diet 200, greatest with 202.

In the liver there were variable numbers of hepatic cells laden with fine fat droplets. With diet 200 this change occurred in isolated cells and in areas alternating with fat-free areas, more often in the midzones of the lobules. Similar changes were present in 1 rat dying after three hundred and fifteen days on diet 220 (also 0.02 per cent DDT). With diets 201 and 202 this fatty change was more extensive and generally involved the periportal halves of the lobules; in addition, a centrolobular cytoplasmic oxyphilia was noted. This varied from a vague diffuse change to segregation of hyaline oxyphilic masses in the central portion of the cell cytoplasm, sharply demarcated against a peripheral basophilic rim and often separated from it by a crescentic or annular vacuole. Two of the 4 rats fed diet 202 (0.1 per cent DDT) presented focal areas of coagulation necrosis of liver cells. In one of these, marginal organization was present, and organizing portal thrombi were identified, as has been noted in high level quinacrine hydrochloride intoxication (Wright and Lillie³; Nelson and Fitzhugh.⁴) In the other rat necrosis was recent, centrolobular, partly hemorrhagic, and variable in amount in the several lobes of the liver.

On the 0.02 and 0.05 per cent DDT diets the adrenal glands appeared normal, while on the 0.1 per cent DDT diet the medulla presented some interstitial phagocytes laden with fat droplets, as well as slight fatty changes in the parenchymal cells.

In regard to the kidney, focal chronic nephritis and pyelonephritis were observed in about half of the rats, without relation to dosage. Usually they were minor in extent, the most severe occurring in a rat whose diet contained 0.02 per cent DDT. Outside of focal areas of atrophy there were some diffuse degenerative changes affecting chiefly the deep or proximal convoluted tubules. The epithelium of these tubules was swollen and finely granular, and its radial striation more or less obscured, especially with the 0.05 and 0.1 per cent DDT levels. Fine fat droplets were present in the base of the epithelium in all rats fed the 0.1 per cent DDT diet, and in one of each of the groups whose diets contained the lower DDT percentages.

The foregoing changes are similar in nature to those previously described by us and by Nelson and co-workers⁵ for rats with shorter periods of exposure.

3. Wright, C. I., and Lillie, R. D.: *Pub. Health Rep.* 58:1242, 1943.

4. Nelson, A. A., and Fitzhugh, O. G.: *Federation Proc.* 3:91, 1944.

5. Nelson, A. A.; Draize, J. H.; Woodard, G.; Fitzhugh, O. G.; Smith, R. B., Jr., and Calvery, H. O.: *Pub. Health Rep.* 59:1009, 1944.

As before,¹ the hepatic alterations in the 10 additional cats studied were chiefly varying grades of fatty degeneration, perhaps more often midzonal in location, and entirely of fine droplet type. Only in 2 cats was patchy cytoplasmic oxyphilia of hepatic cells observed; distinct hyaline globules were not formed, and no cellular necrosis was noted. The periods of exposure of these cats varied from eighteen to sixty-six days at dosage levels of 5 to 50, usually 5 to 10 mg., per kilogram daily.⁶ Several had presented fairly severe tremors for several days or longer before death.

The cats presented about the normal amount of fat storage in renal epithelium, and in about half there was irregular cortical tubular distention with or without oxyphilic foamy or globular exudate in the lumens. In 7 cats the spleen was essentially normal except for slight to moderate

Dosage of DDT, Survival Time and Hepatic Lesions Produced in Rabbits Given DDT by Stomach Tube

Rabbit No.	Dosage	Total Survival Time	Manner of Death	Hepatic Lesions	
				Necrosis	Hyaline Degeneration
20	9 doses of 25 mg. per Kg. = 225 mg. per Kg....	13	Died	—	—
18	1 dose of 400 mg. per Kg.....	15	Died	—	+
206	40 doses of 50 mg. per Kg. = 2 Gm. per Kg....	46	Died	+	—
52	7 doses, then 30 days' rest, then 13 doses of 100 mg. per Kg.....	60	Died	+	—
53	19 doses of 100 mg. and single doses of 240 and 250 mg. per Kg.....	75	Died	++	+
9	2 doses of 400 and 300 mg. per Kg., 2 months' rest, then 1 dose of 400 mg. per Kg. and animal killed 4 days later.....	83	Killed	++	—

* This animal presented incipient cirrhosis.

hemosiderosis in 5 of them. Three of these 5 also showed some siderosis of Kupffer cells. The heart showed fine droplet fatty degeneration of a variable minority of muscle fibers.

The brain and the spinal cord presented pericellular vacuolation about anterior horn cells and sometimes also in motor nuclei of the medulla and higher areas of the brain stem in the 3 cats studied. In one of these, fine fat droplets appeared in many anterior horn cells, and there was partial tigrolysis. This animal had had tremors and progressive paralysis for six days before it was killed.

The liver was studied in 6 rabbits treated with DDT for thirteen to eighty-three days (see table for dosage). The first (no. 20) was without hepatic change, the second (no. 18) presented centrolobular fatty degeneration and hyaline oxyphilic cytoplasmic globules (fifteen days)

6. Smith, M. I., and Stohlman, E. F.: Pub. Health Rep. 60:289, 1945, tables 4 and 6.

and the remaining 4 (forty-six to eighty-three days) had coagulation necrosis of variable extent, both midzonal and centrolobular. The hyaline oxyphilic cytoplasmic inclusions were present in surviving areas in one of these (no. 53). In another (no. 52) sparse fibroblastic trabeculation was developing and was segregating nodules of large basophilic liver cells. The trabeculae included also more numerous foamy (fatty?) liver cells, a little collagen, a few multinucleated giant cells and some hemosiderin-laden phagocytes. Necrosis was distinctly focal in this liver.

The kidneys and the adrenal glands presented no significant changes, the spleen moderate to rather pronounced hemosiderosis, the heart a little patchy fatty degeneration of muscle fibers.

In the hepatic hyaline degeneration and necrosis, this series is similar to those previously studied. The occurrence of an example of apparent incipient hepatic cirrhosis in conjunction with the necrosis is of interest.

DBRDT

Autopsies were made on 8 rabbits after administration of the dibromodiphenyl analogue of DDT, or DBrDT. All died in four to twenty-two days. All presented more or less extensive necrosis of hepatic cells, varying from necrosis of isolated cells only in 2 animals to large anastomosing or confluent areas of coagulative necrosis, to hemorrhagic and fibrinoid variants of the latter and to areas of congested and depleted hepatic reticulum. As with DDT in this species, there were found also, but only occasionally, focal areas of greatly swollen, highly vacuolated, fat-free liver cells. Hyaline oxyphilic cytoplasmic masses surrounded by basophilic cytoplasm, which directly adjoined them or was separated from them by annular and crescentic vacuoles, were also found in liver cells in 4 rabbits. There were also variable amounts of fine droplet fatty degeneration of liver cells. Fatty and hyaline degeneration as well as necrosis tended to occur more in the centers and midzones of the lobules. Leukocytic invasion of necrotic foci was prominent in 3 rabbits but did not involve all foci even in these.

Four of the rabbits presented more or less pronounced congestion of the lung and serous to hemorrhagic exudate in the alveoli. The heart exhibited varying degrees of fine droplet fatty degeneration of muscle fibers. The kidneys presented more or less cloudiness and swelling of the convoluted tubules, often with slight to moderate deposition of fine fat droplets in the bases of the epithelial cells. In some animals the epithelium of loop tubules was also fatty, and in some numbers of fat phagocytes or fibroblasts laden with fine fat droplets were present in the stroma of the renal pyramids.

The spleen regularly showed a moderate amount of hemosiderosis, including both the basophilic and nonbasophilic granular types and in some animals diffuse iron-staining of cytoplasm of both littoral and free

phagocytes. In the liver Kupffer cells were iron free; there was no siderosis of renal epithelium, and hemoglobin casts were not observed.

The adrenal glands were normal, with fatty cortex and intact medulla.

DDD'

Autopsies were made on 8 rabbits which had been given 4,4'-dichlorophenyl-dichloroethylene (DDD'). Six died at five to thirty-two days; 2 were killed at thirty-two days.

The liver presented an inconstant slight to moderate fine fat droplet degeneration of hepatic cells. One rabbit had hyaline oxyphilic masses and globules in the otherwise basophilic cytoplasm of liver cells in the centers of the lobules; another showed foci of hydropic degeneration in which were included some coagulated necrotic liver cells.

The spleen presented moderate to rather striking iron-positive pigmentation of macrophages and littoral phagocytes in the pulp. The pigment was of both the diffuse and the granular type.

In the kidneys there was some deposition of fine fat droplets in the epithelium of corticomedullary straight tubules, while cortical convoluted tubules showed some cloudiness and swelling of the epithelium but no fat.

The heart muscle was normal and fat free in the 2 rabbits killed at thirty-two days, while in 4 others it showed slight to moderate fatty degeneration.

Pulmonary congestion and edema were present in 2 rabbits, empyema in a third and focal perivascular lymphocytic infiltration in the rest. The pulmonary congestion and edema were probably significant; the other changes were almost surely intercurrent.

Focal hemorrhage was present in the adrenal cortex in 1 rabbit (five days), focal karyorrhectic purulent infiltration in another, while in all the animals the adrenal cortex was moderately to heavily laden with lipid substances. The medulla was normal in all.

Two rats dying three and a half days after a single dose of 1.5 Gm. per kilogram presented moderate fatty changes in liver cells, moderate to marked fatty degeneration of renal convoluted tubules with less involvement of loop tubules, moderate and rather extensive fatty degeneration of the heart muscle, much phagocytosed fat and a moderate amount of hemosiderin in the splenic pulp and in the follicular littoral cells, and normal adrenal glands.

DDD

Eight more rabbits were given 2,2-bis-(parachlorophenyl)-1,1-dichloroethane (DDD). In this series 4 rabbits died and 4 were killed at intervals of three to thirty-nine days. The liver presented inconstant and relatively slight patchy fatty degeneration. Foci of necrosis were present

in 3 rabbits, but in one the single lesion was old and encapsulated in fibrous tissue after only three days' exposure; in another a single recent focus of coagulative necrosis was present after a thirty-nine day exposure; in the third the multiple foci graded into epithelioid granulomas. On evaluation of these lesions it is believed that those in the first 2 rabbits may be excluded as not significant; in regard to the lesions in the third the question of significance must be left open.

The renal convoluted tubules were generally cloudy, perhaps swollen, but fat free. Edema of the renal pelvic fat was present in the 4 rabbits which survived over thirty days. Patchy fatty degeneration of the heart muscle was noted in 4 of the 8 rabbits, and in 1 of the 4 there was an organizing area of necrosis. The splenic pulp generally presented impressive siderosis, the pigment occurring more as diffuse iron staining of the cytoplasm of macrophages and littoral cells, but including granular hemosiderin as well, in all animals.

There were congestion and slight edema of the lung in 1 rabbit, edema, purulent bronchitis and focal purulent pneumonia in 1, and no significant pulmonary lesions in 4. The adrenal glands were regularly normal, with lipid depletion of the glomerular zone in 2 of 7. In 1 rabbit a focal area of gastric hemorrhage and edema was encountered; in 1 there was acute ulcerative ileitis. The significance of these gastrointestinal lesions is questionable.

One rat died three and a half days after a single dose of 2.5 Gm. per kilogram and another twelve days after a single dose of 3 Gm. per kilogram. They presented moderate fatty degeneration of the liver, marked fatty degeneration of renal convoluted tubules, much phagocytosis of fat and moderate hemosiderosis in the splenic pulp and follicular littoral cells, some fatty degeneration of medulla cells in the adrenal glands and, in a single rat only, patchy fatty degeneration of heart muscle.

DDM

Autopsies were made on 5 rabbits given nineteen to thirty-two doses of 50 mg. per kilogram of bis-(parachlorophenyl)-methane (DDM) over periods of twenty-two to thirty-seven days. One rabbit died with purulent bronchitis, pulmonary edema, purulent pleurisy and pericarditis and centrilobular fatty degeneration and necrosis of the liver. In the 4 rabbits remaining, the liver presented only slight fatty changes in parenchymal cells and some phagocytosis of fat in littoral cells. The kidneys and the adrenal glands were normal. The heart muscle was usually fat free; 1 rabbit showed involvement of a few fibers. Congestion and edema of the lung occurred in 2 rabbits. The splenic pulp presented definite hemosiderosis both as granules and as diffuse iron staining of the cytoplasm of littoral cells and free macrophages. No gastric lesions were observed.

DT

Autopsies were made on 11 rabbits which had received over periods of two to thirty-eight days aggregate doses of 200 mg. to 1.6 Gm. per kilogram of 2,2-diphenyl-1,1,1-trichloroethane (DT).

In the liver fatty changes were generally slight or lacking. Focal necrosis, coagulative, fibrinous or purulent, was seen in 4 rabbits, 3 dying in two to four days, and 1 in twenty-six days, after daily doses of 100 mg. per kilogram. Oxyphilia of the central part of the cytoplasm of hepatic cells, without segregation of distinct hyaline globules, appeared in 3 rabbits killed or dying on the tenth, tenth and sixteenth days. The 4 animals killed after thirty-one to thirty-eight days showed slight fatty changes as the only hepatic alteration.

The epithelium of the renal convoluted tubules was often cloudy, less often swollen, and in 5 animals only of the 10 studied was there slight patchy fatty degeneration. Edema of the pelvic fat was noted in 2 rabbits.

Focal hemorrhage appeared in the fatty adrenal cortex in 1 of 8 rabbits. In the remaining 7 the adrenal glands were normal.

One rabbit had focal purulent and necrosing fibrinous pneumonia and mediastinitis, fibrinous pericarditis and focal myocardial hemorrhage and edema. In 3 other rabbits pulmonary congestion and edema were noted, while no significant lesions appeared in 3. Slight to fairly prominent focal to diffuse fine droplet fatty degeneration of heart muscle appeared in 6 of 8 rabbits.

The splenic pulp presented moderate hemosiderosis of littoral cells and of free macrophages. The pigment was of both granular and diffuse types in 5 rabbits, granular only in 2 and slight and diffuse only in 1.

One rabbit had subacute ulcerative typhlitis with glandular hyperplasia, hemorrhage, edema, exudation of fibrin and local peritonitis and in one area of the stomach necrosis, edema, hemorrhage and calcification of muscle. In this animal there was also subacute interstitial nephritis. The significance of these lesions in relation to DT is not clear.

DDA

P,p'-dichlorodiphenylacetic acid (DDA) was given intravenously to 6 rabbits, the sodium salt being used in aqueous solution. These rabbits received single doses respectively of 200, 150, 100, 150, 150 and 100 mg. per kilogram and died one and a half, three, four, five and a half, six and seven and a half days later. (The lethal dose [50 per cent] of DDA when it is injected intravenously into rabbits is between 100 and 150 mg. per kilogram.)

In 1 rabbit the liver was normal except for slight hemosiderosis of littoral cells; in 2 there were fairly definite congestion and slight to moderate fatty degeneration of hepatic cells; in 2, isolated hepatic cells,

respectively few and numerous, exhibited cytoplasmic oxyphilia grading over to coagulative necrosis and to the formation of hyaline oxyphilic cytoplasmic inclusion bodies. Of three blocks from the liver of the sixth rabbit, there was confluent or an astomosing midzonal coagulative necrosis of hepatic parenchyma generally in one and focally in another; the third block showed none. In the necrotic areas centers of lobules were sometimes involved as well, the included capillaries were engorged with red corpuscles, and marginal surviving liver cells were laden with fine fat droplets.

The kidney regularly presented irregular dilatation of convoluted tubules and more or less numerous casts within them. The casts were sometimes hyaline and basophilic, more often oxyphilic and hyaline or finely granular, and frequently stained orange pink with eosin in the same tint as red corpuscles within vessels. The last type were hyaline or finely to coarsely granular or even composed apparently of erythrocytes. Pyramidal tubules often contained similar hemoglobin casts. Otherwise, convoluted tubules often presented normal lining epithelium or only focal deposition of fine fat droplets in the epithelium. In the 2 rabbits surviving to six and seven and a half days there were respectively numerous calcified necrotic epithelial cells in the convoluted tubules and a few small concentrically laminated calcareous bodies in the lumens. Albuminuria is a constant finding in rabbits after intravenous administration of DDA.

Three rabbits presented pulmonary congestion and a moderate amount of serous exudate in the alveoli. In the others no significant pulmonary lesions were observed.

The heart presented patchy to diffuse deposition of fine fat droplets in the muscle fibers, sometimes of high grade, in the 5 rabbits surviving three to seven and a half days.

The adrenal cortex was generally fatty in the inner half, and sometimes throughout. The medulla appeared normal.

The splenic pulp generally contained a considerable amount of blood and moderate numbers of lymphocytes. In the rabbit which died in thirty-six hours the iron reaction was negative, while in the rest of the animals considerable amounts of hemosiderin were present in swollen littoral cells and free phagocytes. The follicles were small and relatively inactive.

Six other rabbits received DDA by stomach tube, 50 mg. per kilogram daily. Two of them died at nine and twenty days; 3 were killed on the thirty-eighth day and 1 on the fifty-second.

The rabbit dying at twenty days presented purulent bronchitis and pneumonia, focal purulent and coagulative necrosis in the heart muscle, focal necrosis with marginal organization in the liver, necrosis, regeneration, hyaline casts and slight fatty degeneration in the kidney and marked splenic hemosiderosis.

Otherwise, in this series only slight fine fat droplet deposition was noted in hepatic and sometimes in Kupffer cells. The kidneys showed areas of tubular dilatation and some epithelial or hyaline casts in 4 of the 5 remaining rabbits, usually without fatty changes. Slight to moderate patchy fine droplet fatty degeneration of the heart muscle appeared in 3 of the 5 rabbits. A pulmonary infarct was present in 1 rabbit, bronchitis and edema in 1 and no pulmonary lesions in 3. The adrenal cortex was fatty in all 5; the medulla, normal. The splenic pulp in 3 of 4 rabbits presented moderate pigmentation by iron-positive and often also iron-negative pigment.

Altogether, the rabbits given DDA by stomach tube presented much less striking lesions than those dying after intravenous administration, but blood destruction again seems to have been the principal injury.

Three rats were killed six days after intravenous injection of 100 mg. per kilogram of DDA. They showed no significant lesions of the liver, the adrenal glands or the lungs. Focal renal necrosis was present in 1 rat, and slight cloudy swelling in 1. In all the spleen presented moderate erythropoietic and myelopoietic activity, moderate numbers of megakaryocytes and little or no hemosiderin. All were killed in six days.

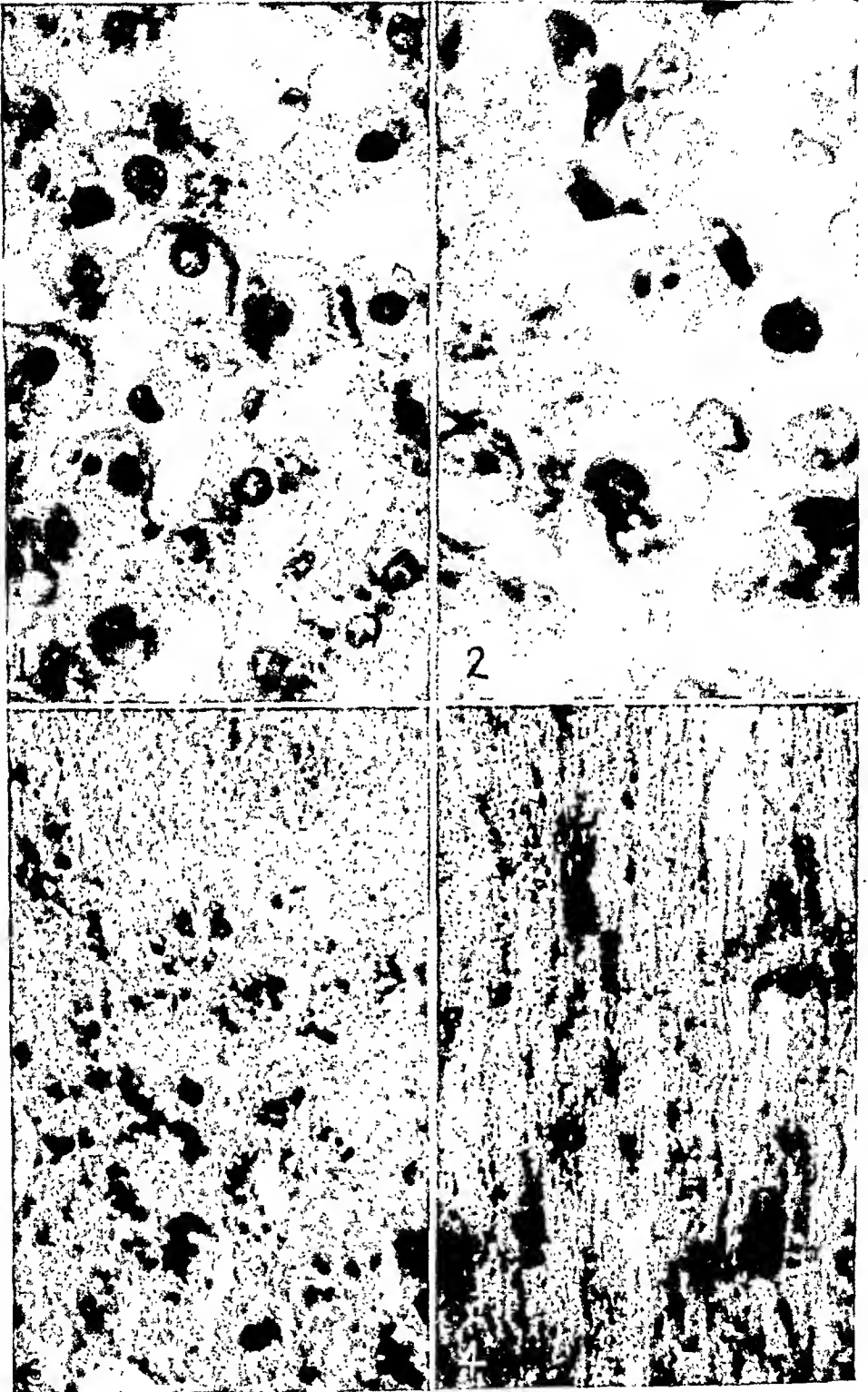
DE

Five rabbits were given twenty-one to thirty-four doses of 50 mg. per kilogram of diphenylethane (DE) over periods of twenty-three to thirty-nine days. Renal convoluted tubules were radially striated and fat free. The adrenal medulla was normal; the cortex heavily laden with lipid. One rabbit had an abscess of the adrenal cortex, probably metastatic from the chronic necrosing pneumonia noted in one lung. Another rabbit presented pulmonary edema, while in 3 there were no pulmonary lesions. Patchy fatty degeneration of the heart muscle was noted in 1 rabbit but not in 3 others. Scattered liver cells and Kupffer cells contained small amounts of fat in fine droplets, and the epithelium of small bile ducts was often moderately laden with fine fat droplets. In 1 rabbit a pyogenic ulcer of the stomach and focal cholecystitis were present. In 4 rabbits the splenic pulp presented moderately severe hemosiderosis, the pigment appearing both in granular and diffuse form in littoral cells and in free macrophages.

DA

Four rabbits were given thirty to thirty-three doses of 50 mg. per kilogram of diphenylacetic acid (DA) over periods of thirty-five to forty-one days.

Three animals presented centrilobular accumulation of coarse and medium fat globules in liver cells, moderate in 2 rabbits and slight in 1. The remaining rabbit showed none. In 1 rabbit examined three hours post mortem, some epithelial desquamation and hyaline casts were found



(See legends on opposite page)

in renal convoluted tubules; otherwise the renal parenchyma was normal and fat free. One rabbit had ulcerative pyelitis with a stone. Fatty degeneration of the heart muscle was present in 3 rabbits, slight in 2, and rather marked in 1; the remaining rabbit showed none. Moderate hemosiderosis of pulp phagocytes and littoral cells was noted in the spleen in all 4. The adrenal cortex was fatty; the medulla, normal. Congestion and serous alveolar exudation were present in the lungs in 2 rabbits but not in the other 2. The stomach and the gallbladder were normal.

DDK' AND DDK

Dichloro-benzophenone was given to 9 rabbits, 6 receiving the asymmetric 2,4'-compound (DDK'), 3 the symmetric 4,4'- or para-, para'-isomer (DDK). Three rabbits received four doses of 400 mg. per kilogram of DDK' and died on the fourth day. Two had an additional 800 mg. and died on the ninth day. One received seven doses of 400 mg. per kilogram and survived ten and a half days.

With the 2,4'-isomer the lungs presented more or less marked congestion and edema. Alveolar exudate was mainly serous and faintly to moderately eosinophilic, and in the animals dying in four days it contained moderate numbers of desquamated rounded epithelial cells. Attached swollen alveolar epithelium was conspicuous in 1 rabbit.

With 4,4'-dichloro-benzophenone there were no pulmonary lesions in 2 rabbits, while lobar pneumonia was found in the third.

With both isomeres the heart exhibited slight to moderate, patchy to diffuse deposition of fine fat droplets in muscle fibers. The adrenal cortex was more or less laden with fat, especially in the inner half, and the medulla was essentially normal. In the spleen slight to moderate hemosiderosis of pulp littoral cells was the principal finding. Iron-positive pigmentation occurred both as diffuse cytoplasmic staining and as typical granular hemosiderin.

The renal convoluted tubules were generally more or less cloudy and swollen. Fatty changes were inconstant and of slight to moderate grade when present. Necrosis and desquamation of epithelial cells were noted in 1 rabbit with each isomere, hyaline casts in dilated tubules in 2 others of each group. In 3 rabbits receiving the 2,4'-dichloro-benzophenone, casts and epithelial necrosis were absent, but orange-staining

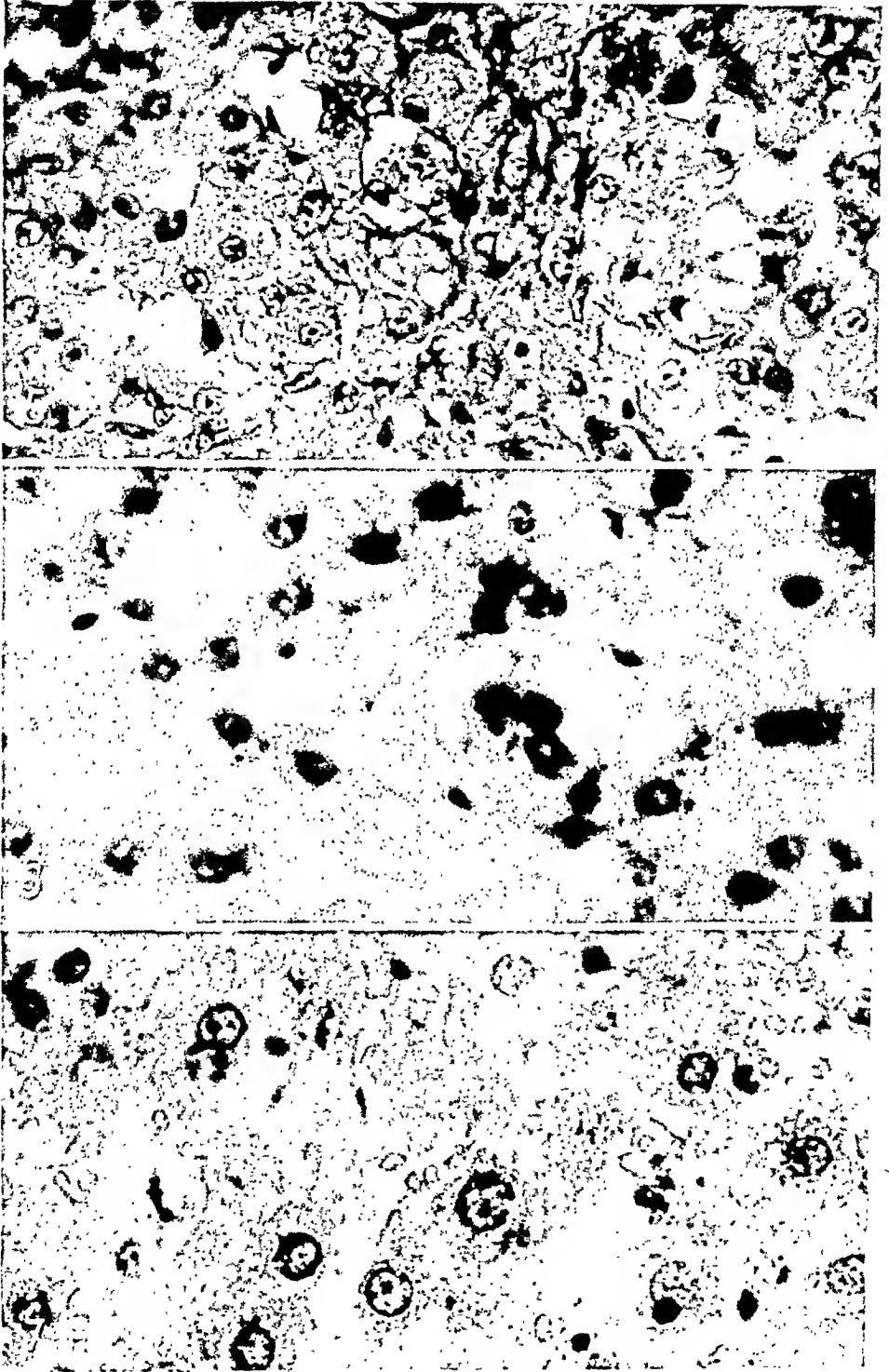
EXPLANATION OF FIGURES 1 TO 4

Fig. 1.—Hyaline liver of a rabbit fed DT for ten days; azure eosin; $\times 700$.

Fig. 2.—Cytophyknosis and necrosis of isolated hepatic cells of a rat fed a single dose of DMDT and killed seven days later; azure eosin; $\times 1,000$.

Fig. 3.—Hemosiderosis of the spleen of a rabbit fed DDD and examined thirty-eight days later; acetic ferrocyanide safranin; $\times 250$.

Fig. 4.—Fatty degeneration of the heart muscle of a rabbit given DDD' and examined thirty-two days later; hematoxylin oil red O; $\times 250$.



(See legends on opposite page)

eosinophilic casts were noted in pyramidal tubules in 1 rabbit receiving this compound. Fine fat droplets had been deposited in the epithelium of the loop tubules in the corticomedullary zone in all 6 rabbits with the 2,4'-isomere and in 1 of the 3 with the 4,4'-compound.

The pathologic alterations of the liver were unfortunately complicated by the presence of definite or probable recent or old coccidiosis in nearly all of these animals. Otherwise minor fatty changes were seen in all the rabbits. The 1 rabbit in which the deposition of fine droplets of fat in liver cells was of severe grade presented also patchy areas of swollen hydropic liver cells enclosing a few isolated coagulated necrotic cells, as in DDT poisoning. This was the latest survivor in the 2,4'-dichlorobenzophenone group.

DMDT

When p,p'-dimethoxydiphenyl-trichloroethane (DMDT) was administered to 9 rats by stomach tube in single doses of 2 to 8 Gm. per kilogram, only 1 rat died, death occurring after five days. This rat received the lowest dose of the group. In this animal the liver presented many isolated hepatic cells in varying stages of coagulative necrosis, from simple cytoplasmic oxyphilia to rounded, shrunken oxyphilic globules with or without contained nuclear fragments. There were also focal areas of engorged and depleted parenchyma, in which there were often isolated surviving and necrosing liver cells, rarely coherent coagulated necrotic liver cells. Fatty degeneration of liver cells occurred irregularly, especially adjoining areas of necrosis. Two other rats, killed on the seventh day, showed necrosis of isolated liver cells. One of these had a considerable amount of epithelioid cell proliferation about necrotic cells and independently, to form small granulomas. Small granulomas were seen also in another rat, centering about clumps of fragmenting leukocytes. These are of dubious significance. Otherwise, the remaining rats showed fatty degeneration of scattered isolated liver cells.

In this same series, a material amount of renal fatty degeneration and of fatty degeneration of heart muscle was evident in the same rat which showed the greatest hepatic damage. In the other rats there was often focal interstitial nephritis, such as one often finds in rats, and small foci of interstitial myocarditis. In 1 rat there was a bulky area of fatty degeneration and atrophy of the heart muscle, with extensive

EXPLANATION OF FIGURES 5 TO 7

Fig. 5.—Destruction of hepatic cells and beginning interstitial fibrosis and trabeculation in a rabbit fed DDT for sixty days; Van Gieson stain; $\times 250$.

Fig. 6.—Hyaline degeneration of the liver of a rabbit given DDD' and examined thirty-one days later; azure eosin; $\times 700$.

Fig. 7.—Necrosis of isolated cells of the liver of a rabbit which died thirty-six hours after receiving NaDDA intravenously; azure eosin; $\times 700$.

interstitial proliferation and lymphoid cell infiltration. This involved the base of the left ventricle. This lesion was regarded as intercurrent.

The lungs presented more or less frequent interstitial and perivascular lymphocytic infiltration, hyperplasia of bronchial lymphoid follicles and

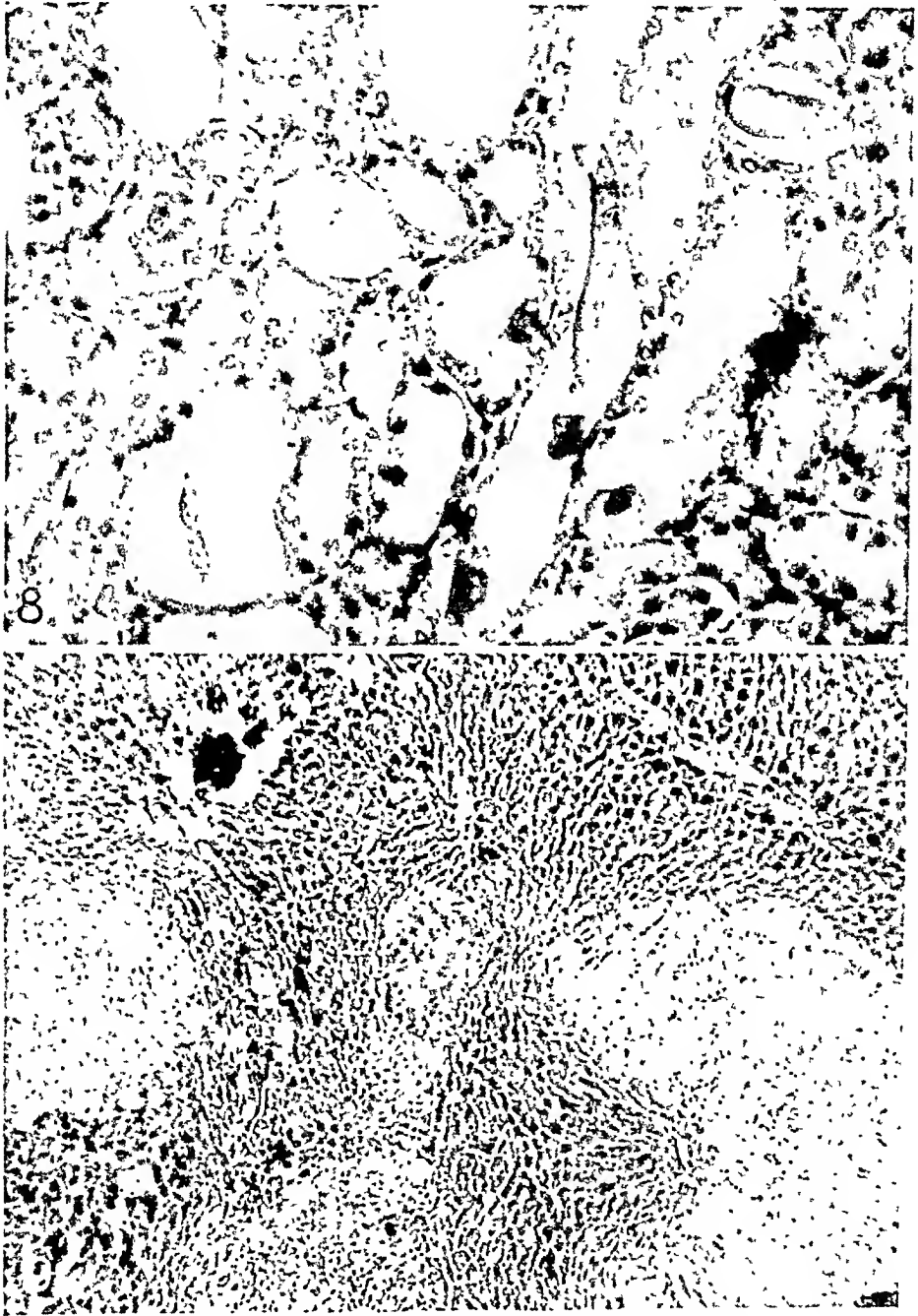


Fig. 8.—Hemoglobin casts in the kidney of a rabbit that died five and a half days after receiving NaDDA intravenously; azure eosin; $\times 250$.

Fig. 9.—Necrosis of the liver of a rabbit which died six days after receiving NaDDA intravenously; azure eosin; $\times 70$.

patchy atelectasis. In 1 rat there was focal organizing bronchopneumonia. Such lesions are not uncommon in rats, especially after feedings by stomach tube.

Moderate splenic myelosis was present in 3 rats, pulpal hemosiderosis in 3 others and some follicular hyperplasia in 2 others. Altogether, there were no consistent alterations.

Four rabbits were given daily doses of DMDT at 200 mg. per kilograms by stomach tube. They received respectively five, six, nine and fifteen doses and died in five, six, ten and twenty days.

The first of these presented ulcerative bronchitis, seropurulent pneumonia and pulmonary edema, also some parenchymatous degeneration of the kidney and fatty degeneration of the heart muscle and of the hepatic parenchyma, with vague areas of hepatic cell oxyphilia and karyopyknosis suggesting incipient necrosis. Pulmonary edema and interstitial pneumonitis, fatty degeneration of the liver and parenchymatous degeneration of the kidney were the principal findings in the second. In the third there were pulmonary congestion, purulent bronchitis and bronchopneumonia, necrosis of the gallbladder and of adjacent liver tissue (but not of other parts of the liver), parenchymatous and fatty degeneration of the kidney with some epithelial necrosis, desquamation and perhaps early calcification, and catarrhal enterocolitis. In the fourth rabbit again there were edema of the lungs, parenchymatous degeneration, epithelial necrosis and desquamation of epithelium of the kidney, slight centrilobular fatty degeneration of the liver and focal myocardial necrosis with calcification. The second and fourth rabbits presented moderate hemosiderosis of the splenic pulp.

The pulmonary changes were probably due to the repeated feedings by stomach tube and consequent accidental aspiration. Otherwise, the renal degenerative changes appear to be the most consistent alterations. The number of animals studied is small, however.

SUMMARY

Further studies of the cat brain and cord in DDT poisoning revealed moderate fatty degeneration of nerve cells as a probably significant, though infrequent, finding. In 1 rabbit incipient trabeculation and nodular hyperplasia of the liver developed on the basis of distinctly focal necrosis, indicating the possibility of hepatic cirrhosis from more prolonged exposures. Nelson and co-workers⁶ have also noted regenerative hyperplasia of the liver in combination with necrosis.

The hepatic lesions induced by dibromodiphenyl-trichloroethane closely resemble those of DDT poisoning: coagulative necrosis and hyaline, hydropic and fatty degeneration. Of these changes, only the fatty degeneration recurred with any consistency with other derivatives, and it was often slight in grade. With DDD and DDD' it was slight and

inconstant, with DDM slight, with DT slight or lacking, with DDA slight after feeding by stomach tube and moderate after intravenous injection, with DE slight, with DA variable, with DDK and DDK' slight to moderate. Focal and patchy necrosis, other than clearly inter-current lesions, were present in 1 rabbit each in the DDD, DDD' and DDK' series, in 4 of the DT series of 11, in all 8 given DBrDT and in 3 of 6 given DDA intravenously. Often these lesions were few, and sometimes they were apparently purulent in character. Their relation to the experimental condition cannot be excluded on the latter account, since hepatic necroses due to DDT are also often heavily infiltrated by leukocytes.

Hyaline degeneration of the cytoplasm of liver cells¹ is probably more definitely a toxic effect. It was found in 3 of 6 DDT rabbits, in 4 of 8 DBrDT, 1 of 8 DDD' and 3 of 11 DDM rabbits. The focal hydropic degeneration previously described in DDT poisoning occurred in 5 rabbits—3 on DBrDT, 1 each on DDD' and DDK'—and the foci, as before, often contained a few coagulated necrotic cells as well.

Other lesions of interest in the present series are the renal and the splenic changes. More or less consistently throughout the series of compounds used, moderate hemosiderosis of the splenic pulp was produced. As a part of the same picture, definite hemoglobinuric nephrosis was produced by DDA when this was administered by the intravenous route. Less pronounced nephrosis, without obvious hemoglobinuria, appeared also in most of the animals given this compound by stomach tube. Otherwise moderate cloudy swelling and fatty degeneration of convoluted and loop tubules appeared with DBrDT—less with DMDT and with DT. There was an instance of hemoglobinuric nephrosis with DDK'. Fatty degeneration of loop tubules was the principal change with DDD, DDD', DDK and DDK', while no renal damage was evident with DE, DA, DDM or DDT in rabbits.

Congestion and edema of the lungs were observed in 4 or 5 lungs studied histologically with DBrDT, 1 of 2 with DDT, 1 of 6 with DDD, 2 of 8 with DDD', 2 of 5 with DDM, 1 of 5 with DDA administered by stomach tube, 1 of 4 with DDA injected intravenously, 1 of 5 with DE, 2 of 4 with DA, 4 of 7 with DT, all of 6 with DDK' and none of 3 with DDK. Purulent pneumonia was seen in 4 rabbits which had received respectively DDD, DDD', DE and DDK. All rabbits given DMDT had either pneumonia or pulmonary edema.

It is necessary to consider aspiration secondary to feeding by stomach tube as a possible factor in the causation of these pulmonary changes.

REPAIR IN THE SKIN OF GUINEA PIGS SUBSEQUENT TO APPLICATIONS OF 20-METHYLCHOLANTHRENE

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AND

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THAT the skin of guinea pigs is resistant to the action of polycyclic hydrocarbons is manifest in the low incidence of cancers following administration of these substances. Fibrosarcoma and liposarcoma have been obtained in the subcutaneous tissues.¹ Carcinoma has not as yet been produced. This does not preclude the possibility that the epidermis of the guinea pig may be able to react in some way to the polycyclic hydrocarbons applied. In mice, prolonged cutaneous application of benzpyrene and methylcholanthrene previous to the making of a wound caused increased proliferation of the epidermis during repair. However, this intensified growth was not accompanied by more rapid epithelization of the defects; on the contrary, the regenerating epithelium was delayed in its migration over the surface of the wound.² The present investigation was undertaken in order to determine whether similar effects might be observed in the repair of the skin of the guinea pigs. Moreover, we intended to study the response of the carcinogen-treated epidermis in different sites, varying in thickness, such as those found in the flank and the ear, in the same guinea pig.³

MATERIAL AND METHODS

Thirty-two male guinea pigs weighing 180 to 220 Gm. at the beginning of the observation were used. The right ears and the right flanks of all animals served as test areas. The hair was carefully clipped before the painting was started, and clipping was repeated at frequent intervals, whenever the hair had

This investigation was aided by the David May-Florence G. May Fund.

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regrown. Three times weekly the skin was painted with a 0.3 per cent solution of 20-methylcholanthrene dissolved in benzene, the solution being applied in one stroke with a no. 6 camel's hair brush. The left ears and the left flanks served as controls. In 16 of the animals the left ear and the left flank remained untreated, to be used later for the making of control wounds. In the 16 remaining guinea pigs the left flank and the left ear were painted with benzene alone for the same periods as the opposite side with methylcholanthrene. Painting was continued for one-half month, one month, two months or three months. After each of these periods wounds were made in subgroups of 8 animals. An area 4 mm. in diameter was marked with a round stencil, and the skin, with some underlying tissue, was excised. Thus in each animal four wounds were made, one in each ear and one in each flank. On the right side each guinea pig had two wounds which were under the influence of the previously applied carcinogen. On the left side each animal carried control wounds, made either in normal skin or in epidermis previously treated with benzene. Repair was allowed to take place for three, five, eight or ten days. At the end of each experimental period the wounds, with the surrounding skin and subcutis, were removed, fixed in solution of formaldehyde, U.S.P., diluted 1:10, embedded in paraffin, cut serially and stained with hematoxylin and eosin.

The epithelium at some distance from the line of excision (distant epithelium), that at the margin of the wound (marginal epithelium) and the newly formed epithelium growing in a tongue-like fashion into the defect (new epithelium) were studied separately. In each case the lengths of the epithelial tongues were measured. The size of the cells in each of the various areas was determined, and the epithelial cell layers and the mitoses in the epidermal cells were counted.

GROSS OBSERVATIONS

Only in a few animals after application of methylcholanthrene for three months was some epilation noted. During the operation the wounds made in the areas previously treated with benzene or methylcholanthrene bled more profusely than those made in untreated skin.

HISTOLOGIC OBSERVATIONS

Closure of the Wounds.

All wounds, whether made in untreated or in painted epidermis in the ear or in the flank, were closed eight days after excision; still, the different areas of the epithelium, designated as distant, marginal and new, could be recognized as such, although the tongues had met in the center of each of the defects. At three or five days after operation the tongues varied in length considerably. No definite correlation could be established between the various experiments and the progress of epithelization. Shorter epithelial tongues usually were present if there was marked edema or cellular infiltration in the dermis or the subcutis.

Changes in the Wound Base.

Untreated Animals.—After three days of healing, the connective tissue at the base of the wound was loose, and the uppermost layer was covered with fibrin and slightly infiltrated by polymorphonuclear and

mononuclear leukocytes. Five or eight days after the making of the wound, capillaries advanced toward the surface and many fibroblasts appeared, whereas round cells and polymorphonuclear leukocytes became less numerous. Here and there a multinucleated giant cell was found. After ten days, when the wounds had closed, the tissue became fibrillar, with no evidence of an inflammatory reaction. In the ear and the flank the conditions were similar except that collagenous fibers were much more abundant in the subcutis of the flank than in that of the ear.

Benzene-Treated Animals.—After painting with benzene for one-half month or one month, the connective tissue was looser and more engorged than that in the untreated animals. This change was noted as early as three days after excision, not only in the wound base proper but also in the adjoining dermis and subcutis. The fibrils were thickened. At the floor of the defect were minute hemorrhages and much fibrin; polymorphonuclear and mononuclear leukocytes were more numerous than in the nontreated guinea pigs, and they infiltrated the deeper layers of the subcutis. After five or eight days, numerous engorged capillaries, accompanied by fibroblasts and a few multinucleated giant cells, advanced toward the surface. Polymorphonuclear leukocytes and lymphocytes were scanty. After ten days a densely fibrillar connective tissue was found in the former wound base. As in nontreated skin, the collagenous fibrils were by far more abundant in the flank than in the ear. The changes described were intensified after two or three months of treatment. The swelling of the fibrils was more marked and the exudate was more abundant than after shorter periods of painting. Ten days after operation the collagenous fibers were frequently fragmented. Again, as in the untreated animals, the scar in the flank contained much more collagenous tissue than that in the ear.

Methylcholanthrene-Treated Animals.—After one-half or one month's treatment conditions in both the ear and the flank were comparable to, but more pronounced than, those seen after application of benzene alone; there were also some eosinophils and mast cells. After two or three months of painting with methylcholanthrene the changes in the connective tissue were still similar in kind but more severe than those in the corresponding benzene-treated animals. After ten days the scar was deeper, more collagenous and more vascular and still contained some inflammatory cells.

Thickness of the Epidermis.

In the untreated skin of both the ear and the flank the proportion of basal to spinous cells was 2 or 3 to 1. Under the influence of benzene, as well as under that of methylcholanthrene, this ratio shifted gradually in favor of the spinous cells. Thus, after three months of

painting there was one spinous cell found for each basal cell. As soon as the painting was discontinued, the number of spinous cells decreased again, and ten days after the making of the wound, that is, eleven days after the last painting, the usual ratio of the two cell types was restored in most instances. In table 1 the numbers of cell rows of the epithelium of the ear are presented separately from those of the flank. The data for the untreated skin are shown in columns 2 to 4. The findings after painting with benzene for one-half or one month were similar, as were those after painting for two or three months. The same was true for the figures obtained with methylcholanthrene; therefore, the one-half and one month stages (columns 5 to 10), and the two and three month stages (columns 11 to 16), are grouped together. The numbers of cell rows for the distant, the marginal and the new epithelium are given in individual columns.

Ear.—The untreated epidermis consisted of four rows of cells. During wound-healing the thickness of the marginal epithelium increased to a maximum of nine rows of cells five to eight days after excision; after the wounds were closed, it declined slowly, so that ten days after operation there were seven layers of epithelial cells at the wound margins. During the first five days the advancing epithelial tongues at their insertion were about as thick as the distant original epithelium consisting of four rows of cells. After the defects closed, the tongues were composed of six layers of cells, and they merged invisibly with the old epithelium at the line of excision.

After benzene had been applied for one-half or one month, the epidermis became slightly thickened and contained about five rows of epithelial cells. After excision of a piece of skin the thickness of the epidermis at the wound margin was not increased over that seen in untreated skin. The maximum number of epithelial cell layers was nine. However, in the benzene-treated animals the greatest thickness was present as early as three days after the making of the wound, whereas in untreated epidermis the maximum number of cell rows was seen after five days. As in the untreated skin, the height of the regenerating epithelial tongues was about the same as that of the distant epithelium, and it decreased somewhat as the tongues from both sides joined to close the defect. Treating the epidermis with benzene for two or three months did not produce any further thickening as compared with that seen after one-half or one month's application. But after excision the height of the marginal epithelium increased to ten rows and, in contrast to that of the animals treated for shorter periods, it had a tendency to increase even after the wounds had closed. The same tendency was noticeable in the epithelial tongues.

After one-half or one month's treatment with methylcholanthrene five epithelial cell layers were present in the original (distant) epithelium. At the wound margin the maximum number of cell rows was nine, as

TABLE 1.—Number of Cell Rows in Various Areas of the Epithelium After an Excision Made in Untreated Skin and in Skin Painted with Benzene or with Methylcholanthrene Dissolved in Benzene.

Days of Healing	Untreated Skin			Benzene ¼ or 1 Month			Methylcholanthrene ½ or 1 Month			Benzene 2 or 3 Months			Methylcholanthrene 2 or 3 Months		
	Distant Epithelium	Marginal Epithelium	New Epithelium	Distant Epithelium	Marginal Epithelium	New Epithelium	Distant Epithelium	Marginal Epithelium	New Epithelium	Distant Epithelium	Marginal Epithelium	New Epithelium	Distant Epithelium	Marginal Epithelium	New Epithelium
3	4	6	4	5	9	5	5	9	5	5	8	5	6	9	5
5	4	9	4	5	8	4	5	9	5	4	9	5	5	10	5
8	4	9	5	5	7	5	5	9	5	5	9	6	5	10	8
10	4	7	6	5	6	4	5	8	6	5	10	7	5	8	6
Ear															
3	2	6	4	4	7	5	4	7	5	4	7	4	5	7	5
5	3	7	4	3	8	4	4	8	4	4	7	5	5	9	5
8	2	7	6	4	7	4	4	8	5	4	7	6	5	8	6
10	2	6	5	5	5	5	3	5	5	3	6	3	4	6	5
Flank															

TABLE 2.—Number of Mitoses in Multiples of the Normal in Distant, Marginal and New Epithelium After an Excision Made in Untreated Epidermis and in Skin Painted with Benzene or Methylcholanthrene

Days of Healing	Untreated Skin			Benzene ¼ or 1 Month			Methylcholanthrene ½ or 1 Month			Benzene 2 or 3 Months			Methylcholanthrene 2 or 3 Months		
	Distant Epithelium	Marginal Epithelium	New Epithelium	Distant Epithelium	Marginal Epithelium	New Epithelium	Distant Epithelium	Marginal Epithelium	New Epithelium	Distant Epithelium	Marginal Epithelium	New Epithelium	Distant Epithelium	Marginal Epithelium	New Epithelium
3	1	6	1.5	1.5	6	2	1.5	6	3	2	7	3	1.5	7.5	3
5	1	5	5	1.5	6	4	1	5.5	4.5	1.5	4	4	2	7	6.5
8	1	4.5	3	1	5	2	1	4.5	3	2	3.5	3.5	1.5	6	5.5
10	1	2	1.5	1	2	1	1	2	1.5	1.5	2	1.5	1.5	3	3
Ear															
3	1	3.5	1	1.5	5	2	2	5.5	2	1.5	8	2	1.5	8	3
5	1	6	5	1.5	6	6	1	5	7	2	6	6	2	8	6
8	1	4	3.5	1	3	1.5	1	3	3	1.5	4	3	1.5	4	1
10	1	2	1	0.5	0.5	0	1	1	1	1.5	1.5	1	1	1.5	1
Flank															

in the benzene series; but this "high" remained present for eight days, and there was a slight decline to eight rows of cells after ten days of healing. The epithelial tongues again were of about the same thickness as the distant epithelium. In some of the animals treated for two or three months the number of cell rows in the distant epithelium increased to six. Under the combined stimulation of the carcinogen and the wound the marginal epithelium was not higher than under the influence of benzene and the wound; but the maximum of ten layers of epithelial cells was reached after five days, and it persisted through the eighth day. The epithelial tongues showed, as in the benzene-treated and in the untreated skin, about the same height as the epithelium distant from the wound (five or six rows).

Flank.—The untreated epidermis distant from the incision had two to three rows of cells, compared with four in the ear. During the course of repair the largest number of cell rows seen at the wound margin was seven (five or eight days after operation); the new epithelium was composed of four to six rows of cells. One-half or one month's, as well as two or three months' treatment with benzene increased the number of cell layers in the distant epithelium to four or five. The marginal epithelium around the wounds made in benzene-treated skins reached a maximum thickness of eight rows of cells, and there was little difference between the groups treated with benzene for short or longer periods. The height of the new epithelium slightly exceeded that of the distant epithelium, reaching a maximum of five rows after one-half or one month and a maximum of six rows after two or three months of application. The thickness of the epidermis after one-half or one month's treatment with methylcholanthrene did not differ from that seen after application of benzene alone. Prolonged painting with methylcholanthrene evoked a reaction slightly more marked than that following the same length of application of benzene alone. The difference amounted to one row of cells in the distant, and to two rows of cells in the marginal, epithelium. The maximum of nine cell rows was present after five days, after which time the epithelium again became thinner.

Size of the Epithelial Cells.

Ear.—In the normal skin the basal cells measured 6 to 7 microns in width. Three months' application of the carcinogen increased their diameter to 7 or 8 microns. At the margin of a wound made in untreated skin the diameter of the cells was about 8 microns, and in the new epithelium cells measuring as much as 10 microns were observed, particularly during the first five days of healing. The same increase in size, with some variation, was observed in the cells under the influence of benzene and methylcholanthrene. Here and there the regenerating cells reached a diameter of 12 microns. Thus the effect of the carcinogen on cell size was far less accentuated than that of the excision.

Flank.—The basal cells were normally somewhat larger than those of the epidermis of the ear, having a width of 7 to 8 microns, compared with 6 or 7 in the latter. But under the stimulation of either the wound alone or the wound in combination with methylcholanthrene the maximum diameter did not exceed that reached under the same conditions by the naturally smaller cells of the ear.

MITOTIC COUNTS ON THE EPITHELIUM

In table 2 the numbers of mitoses in 2,000 epithelial cells are presented for the ear and for the flank. The values are means established on the basis of counts of 20,000 cells each in the distant, the marginal or the new epithelium. The number of mitoses in untreated skin was 3 or 4 in 2,000 cells for the ear and 2 or 3 in 2,000 cells for the flank.

The increases of mitoses are presented in multiples of the normal number, so as to allow a better comparison of the counts arrived at under the varying experimental conditions and in the two locations studied. In table 2 the same arrangement is followed as in table 1, and the results obtained after one-half or one month and those established after two or three months are grouped together. The effects of the one-half or one month's treatment were slight at best and do not warrant separate discussion. The changes in the mitotic counts after two or three months' painting were, on the whole, more marked than after short treatment and are illustrated in charts 1 and 2; they will presently be described.

In chart 1 graph *A* gives the mitotic count of the original epithelium at some distance from the line of excision as found for normal animals, for those painted with benzene and for those painted with methylcholanthrene. The maximum count obtained after treatment with either substance was about twice the normal, no difference being noticeable between the effects of benzene and methylcholanthrene. The values are, however, slightly higher than those found after painting for one-half or one month.

In graph *B* are given the mitotic counts made at the margin of the excision. In each case the peak of mitoses was seen three days after the making of the wound. Painting of the skin with benzene or methylcholanthrene for two or three months raised the maximum count to seven or seven and one-half times the usual, respectively, compared with six times in untreated skin. During the later stages of repair the mitotic activity declined. Under the influence of benzene this decline was rapid; it progressed more slowly in the methylcholanthrene-treated skin and after ten days was three times the normal, compared with twice the normal in both the nonpainted and the benzene-treated epidermis.

Graph *C* shows the mitotic counts of the new epithelium. As in untreated skin, the number of mitoses rose up to the fifth day after the

excision and then returned to lower or even normal values. The peak of the mitoses (six and a half times normal) seen after treatment with methylcholanthrene was one and a half times higher than that in untreated epidermis (five times the normal) and two and a half times higher

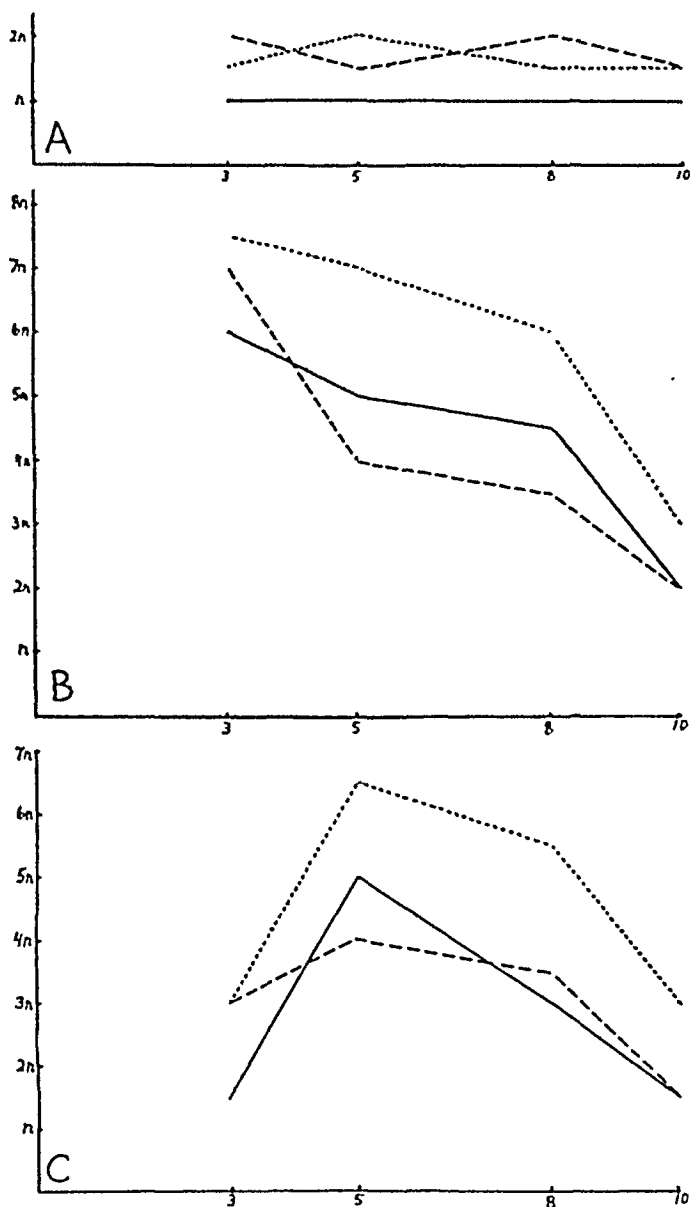


Chart 1.—Mitotic cycles in the epidermis of the ear during ten days of wound-healing of untreated skin (straight line), of skin treated with benzene for two or three months (broken line) and of skin treated with 20-methylcholanthrene for the same periods (dotted line). The mitotic counts are demonstrated in multiples of the normal (n) as designated on the ordinate. The number of days of treatment is shown on the abscissa. *A*, epithelium distant from the wound. *B*, marginal epidermis. *C*, new epidermis.

than that seen after application of benzene (four times the normal). Moreover, as in the marginal epidermis, the number of mitoses declined

more slowly than in either the untreated or the benzene-treated skin, and it was still three times the normal ten days after operation, compared with one and a half times the normal in both the latter.

In chart 2 graph *A* illustrates conditions in the epithelium of the flank at a distance from the wound margin. The highest mitotic count obtained was twice the normal, and it occurred after treatment with either

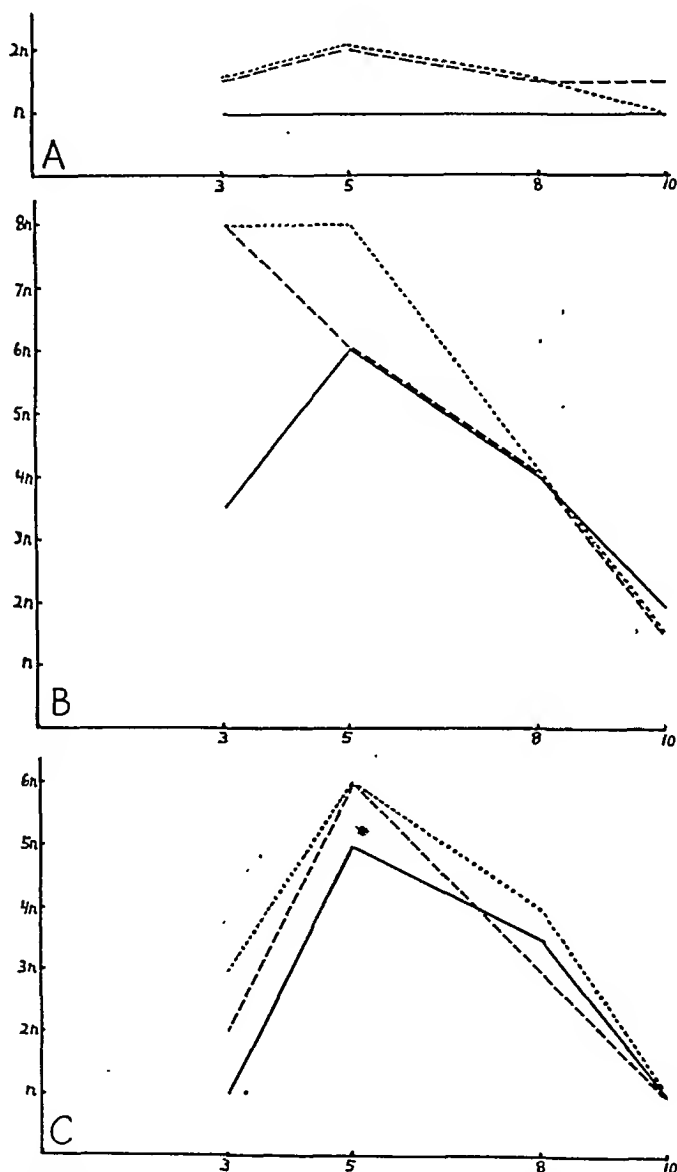


Chart 2.—Mitotic cycles in the epithelium of the flank during ten days of wound-healing of untreated skin (straight line), of skin treated with benzene for two or three months (broken line) and of skin treated with 20-methylcholanthrene for the same periods (dotted line). The mitotic counts are demonstrated in multiples of the normal (n) as designated on the ordinate. The number of days of treatment is shown on the abscissa. *A*, epithelium distant from the wound. *B*, marginal epithelium. *C*, new epithelium.

of the two substances. The mitotic counts declined at similar rates during the later stages of healing.

Graph *B* demonstrates the mitotic counts of the epithelium at the margin of the wound of the flank. The maximum of mitoses was present after three or five days of repair. After painting with either benzene or methylcholanthrene the mitotic count reached a high of eight times the normal, whereas in the untreated skin it was six times the normal. During the later stages of healing the fall in the number of mitoses was rapid, and ten days after the making of the wounds a return to values one and a half times or twice the normal had occurred in all groups.

Graph *C* shows the mitotic counts of the new epithelium of the flank. The three curves took a similar course. They reached their peak five days after excision; the maximum was six times the normal after treatment with benzene or with methylcholanthrene, compared with five times the normal in the nonpainted epidermis. Ten days after the operation the number of mitoses had dropped to normal.

COMMENT

In untreated skin of the ear and the flank of the guinea pig, wounds 4 mm. in diameter were epithelized eight days after operation. The measurements and counts obtained in the present experiments are in agreement with those established in the early work of Loeb and his collaborators on wound-healing in the epidermis of this species.³

Under the influence of either benzene or methylcholanthrene there were no changes in the time required for epithelization of the defects. There was an insignificant increase of epithelial proliferation at some distance from the wound after one-half or one month of painting, but epithelial growth was intensified after two or three months of application of either substance. The mitotic proliferation was of the same order in the benzene-treated and in the methylcholanthrene-treated skin, but the epidermis was thicker in the latter group. The same conditions were noted at the margin of the excision. Here, also, the increase in mitotic activity was negligible after shorter treatment, but became more obvious after prolonged painting. The proliferation as well as the thickness of the epithelium seen in the methylcholanthrene groups exceeded somewhat those occurring under the influence of benzene alone. Similarly in the new epithelium, both the number of epithelial cell rows and the mitotic count were somewhat higher in the skin treated with methylcholanthrene. Thus there was a slight but definite superiority of methylcholanthrene as compared with the solvent benzene as regards stimulation of epidermal growth. In the connective tissue benzene and particularly methylcholanthrene caused enhanced vascularization, edema and increased formation of collagen, which after prolonged treatment underwent fragmentation.

A comparison of the ear and the flank with respect to wound repair gave the following results: In both areas the defects were closed eight days after excision in spite of the differences in the thickness of the epidermis and the number of mitoses seen normally in these two regions. The epidermis of the ear increased in thickness by about one-fourth after being treated with either benzene or methylcholanthrene; in the flank it was almost doubled in thickness. Under the influence of both painting and the wound, the epithelium of the ear increased two and a half times, whereas an increase of three and a half times the normal was seen in the flank. Thus, under the influence of the carcinogen the thinner epidermis of the flank increased in thickness more markedly than the thicker epidermis of the ear. The maximum number of cell rows seen in both locations was five. This seems to indicate that there are limits to the thickening of the skin. Once a certain number of cell rows has been reached, further increase becomes difficult; instead, the superficial layers undergo keratinization and are sloughed off. On the other hand, the mitoses did not show this limitation. The absolute number of mitoses in a given number of basal cells was higher in the ear than in the flank. Their relative increase occurring under the combined stimulus of the wound and the carcinogen was slightly higher in the ear than in the flank. This difference manifested itself particularly during the later stages of healing, when the mitotic counts returned to normal somewhat more slowly in the ear than in the flank.

As compared with the skin of the mouse, the epidermis of the guinea pig showed only a slight reaction after the application of benzene. Both species responded with intensified proliferation of the epithelium. In the mouse, this stimulation was already noticeable after two weeks of treatment, and it was sufficient to accelerate wound healing. In the guinea pig, a noteworthy stimulation of epithelial growth was seen only after two or three months of painting, but it was not enough to hasten the epithelization of the wounds. A difference in the response of the skin of the two species was present as early as one-half or one month after the start of treatment with methylcholanthrene, but it was more conspicuous after prolonged painting. Epilation was a rare occurrence in guinea pigs, and it affected only small, scattered areas of the epidermis. On the other hand, epilation occurred in most mice and extended all over the painted area. In the latter species, the mitotic count of the original epithelium was increased seven or eight times over the normal, and tumors developed during the period of painting. In the guinea pig, the number of mitoses in the original epithelium did not rise above twice the normal, and it is therefore not surprising that neoplasms did not develop. Moreover, in the healing wounds of guinea pigs the period of intensified mitotic activity was not appreciably prolonged as in mice, nor was there any inhibition of the migration of the epithelium

covering the defect as seen in the latter species under corresponding conditions.² Thus the change in the skin of the guinea pig remained restricted to mild hyperplasia, with only slight modifications of the processes of repair, effects partly attributable to the solvent benzene and slightly intensified by methylcholanthrene. In the mouse, by contrast, the true carcinogenic effect of methylcholanthrene manifested itself when this change gradually assumed neoplastic aspects, which coincided with marked disturbances in the epithelization of the wounds.

The reasons for the guinea pig's resistance to carcinogenic substances are unknown. Howes⁴ assumed that methylcholanthrene placed into the subcutis of the guinea pig does not destroy the polymorphonuclear leukocytes, which absorb the carcinogen and remove it, whereas in the mouse these cells are destroyed by the carcinogen, which thus can exert its effect on the tissues. However, in the present experiments the methylcholanthrene was applied directly to the skin, where leukocytes do not so readily accumulate as in the subcutis. This makes it probable that the resistance of the guinea pig is based not only on the activity of the leukocytes but also on as yet unknown properties of the epithelium itself. The failure of the hair to fall out under the influence of the carcinogen suggests the possibility that the latter may not be absorbed by the sebaceous glands as it is in the mouse.⁵ That the age factor plays any role in the resistance of the guinea pig seems unlikely, since five years' continuous cutaneous application of dibenzanthracene failed to produce tumors.⁶

SUMMARY

Painting of the skin of guinea pigs with 20-methylcholanthrene dissolved in benzene or with benzene for periods of two or three months causes slight hyperplasia of the epithelium. The normally thinner epithelium of the flank responds to the painting with a greater increase in thickness but with relatively no greater mitotic activity than the naturally thicker epithelium of the ear.

Treatment with benzene or methylcholanthrene does not alter the time required for the epithelization of dermal defects in guinea pigs as it does in mice. However, the proliferation of the regenerating epithelium is somewhat stimulated after prolonged treatment with either benzene or methylcholanthrene. Methylcholanthrene exerts a slightly more marked stimulation than benzene.

The greater resistance of the epidermis of the guinea pig as compared with that of the mouse subjected to the influence of carcinogenic hydrocarbons thus manifests itself also in the diminished effect of the latter on wound healing.

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Case Reports

HISTOPLASMOSIS IN INFANCY

The Pathologic Picture as Seen in One Case

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SINCE Darling's first article,¹ in 1906, describing the disease which he later called histoplasmosis, 88 cases have been reported in the literature.² Twenty-eight of the patients were children. Twenty were infants less than 15 months of age. Because in few of these cases was histoplasmosis diagnosed clinically and because the case which we have observed presents some interesting features, a report with a complete pathologic and bacteriologic description may be of value. A discussion of the clinical features as seen in infancy will be published elsewhere.³

REPORT OF CASE

A girl was born in Illinois on April 7, 1945, after a full forty weeks' gestation. The infant weighed 7 pounds 8 ounces (3,402 Gm.) at birth and was in normal health until June 1945, when she was seen by the family physician because of irritability and sleeplessness. She responded well to iron therapy.

In October 1945, at 6 months of age, the patient again was taken to the family physician because of icterus, pallor and a temperature of 103 F. Examination revealed hepatosplenomegaly, rales and decreased breath sounds on the left side of the thorax posteriorly. At this time the concentration of hemoglobin was 7.5 Gm. per hundred cubic centimeters of blood; the erythrocytes numbered 2,760,000 and the leukocytes 3,900 per cubic millimeter of blood, with a normal differential count. The cerebrospinal fluid showed no abnormality. A roentgenogram of the thorax revealed no change from normal. Chemotherapy and antibiotic therapy were instituted without avail.

On November 13 the patient was referred to the Mayo Clinic and was admitted to a hospital for further study and treatment. Physical examination on admission to the hospital showed a well nourished, well developed pale infant with moderate hepatosplenomegaly.

The concentration of hemoglobin was 12.6 Gm. per hundred cubic centimeters of blood; the erythrocytes numbered 3,640,000 and the leukocytes 1,900 per cubic millimeter of blood. Blood smears showed hypochromic, macrocytic anemia, with increased regeneration and occasional basophilic stippling. The concentration of serum bilirubin was in the normal range; the cephalin flocculation reaction was positive; the sulfobromophthalein test showed retention, grade 1 (on the basis of

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1. Darling, S. T.: J. A. M. A. **46**:1283, 1906.

2. For a review of the literature see article by A. M. Iams, M. M. Tenen and H. F. Flanagan in the American Journal of Diseases of Children (**70**:229, 1945).

3. Iams, A. M., and Keith, H. M.: Histoplasmosis in Infancy: Report of a Case in an Infant with a Brief Clinicopathologic Review, unpublished data.

1 to 4, in which 1 designates the least and 4 the greatest retention). The concentration of proteins was 5.8 Gm. per hundred cubic centimeters of serum, with an albumin-globulin ratio of 3.06:1. Roentgenograms of the thorax, the head and the long bones were normal.



Fig. 1.—(a) Heart showing perivascular accumulation of phagocytes, beginning fibroblastic proliferation and destruction of the wall of an arteriole ($\times 130$). (b) Lung showing thickened alveolar walls with phagocytes in the alveolar spaces ($\times 150$).

The patient's course was stormy. The rectal temperature ranged daily from 99.6 to 104 F., with peaks at 8 a. m. and 8 p. m. Repeated studies of the blood showed progressive hypochromic macrocytic anemia. Because of the history, the physical findings, the anemia and the leukopenia, histoplasmosis was suspected,

and a biopsy of bone marrow was done on November 23 by Dr. Hargraves, of the Division of Medicine, Mayo Clinic. Typical organisms of *Histoplasma capsulatum* were found in neutrophils, monocytes, reticuloendothelial cells, eosinophils and megakaryocytes, as well as free in the smear itself.

Dermal tests were made by applying histoplasmin to the infant's forearms in dilutions of 1:1,000, 1:500, 1:100 and 1:10 with proper controls. There were no significant reactions in forty-eight and seventy-two hours. A similar dermal test was made on the forearm of the patient's mother, with a positive reaction in forty-eight hours, the erythematous area measuring 5.4 by 3.6 cm. The erythematous area of the control reaction measured 2.4 by 1.6 cm.

Cultures of blood, sternal marrow, stools and duodenal contents all showed *H. capsulatum* in five to twenty days. Repeated cultures of urine were negative.

While in the hospital, the patient received penicillin, repeated blood transfusions and Neostam (the nitrogen glucoside of sodium para-aminophenyl-stibonate) without any appreciable effect. Her condition became progressively worse and she died on December 16.

Because of the positive reaction to the histoplasmin dermal test, the patient's mother was examined for signs of latent histoplasmosis. The examination failed to reveal anything abnormal.

Necropsy.—The body was that of an 8 month old white girl measuring 72 cm. in length and weighing an estimated 20 pounds (9.07 Kg.). There were numerous petechiae scattered over the abdomen and the thorax.

The heart weighed 55 Gm. The epicardium, the endocardium, the valve leaflets and the myocardium appeared grossly normal. Sections for histologic examination showed diffuse interstitial proliferation of fibroblasts and macrophages with a moderate number of infiltrating lymphocytes and plasma cells. There was a tendency for such regions of reaction to be more prominent in the vicinity of the blood vessels. The macrophages contained varying numbers of spherical bodies characteristic of the yeast form of *H. capsulatum*. Although the reaction was primarily between the muscle fibers, there were many regions in which the fibers were abruptly interrupted. The cross striations and the muscle nuclei appeared normal right up to the frayed ends, suggesting that the fibers had been destroyed in the process (fig. 1 a).

The visceral pleura of the lungs contained many petechiae. The lungs were pale pinkish red and firm with an increased consistency resembling organizing pneumonia. They were so firm that after removal from the thorax they retained their original size and shape. The palpable air-containing tissue was confined to the anterior borders adjacent to the pericardium. The cut surfaces were uniformly pale grayish pink, and from them no demonstrable exudate could be scraped. The regional lymph nodes were enlarged, discrete, firm, pale pink and moist on the cut surface. Histologically, the lungs showed extensive proliferation of fibroblasts, with thickening of the alveolar walls, producing diffuse organizing pneumonia. The over-all reaction was that of diffuse proliferation with a minimal amount of exudate (fig. 1 b), and occasional local regions somewhat suggestive of tubercles were found. Indeed, the proliferative reaction was so prominent that it was usually difficult to find the etiologic agent in the routine sections stained with hematoxylin and eosin, although cultures proved the organisms to be present in large numbers.

The liver weighed 556 Gm. and was light brown mottled with yellowish brown areas approximately 1.0 cm. in diameter. The anterior border was rounded. The capsule was smooth. The organ was uniformly firm and the consistency

moderately increased. The cut surface was relatively dry and reddish brown mottled with yellowish brown areas ranging from 1.0 to 2.0 cm. in diameter. Histologically, there were foci of necrosis showing macrophages filled with organisms and extensive fibroblastic proliferation without significant leukocytic infiltration. The periportal regions showed huge numbers of macrophages engorged with yeast forms. In these regions early fibrosis was extensive. The Kupffer cells were laden with organisms and a finely granular material. Many blood vessels were filled with macrophages distended with phagocytosed yeastlike bodies. In many regions these masses appeared to form emboli. There were regions of necrosis in the walls of some of these vessels (fig. 2).

The spleen weighed 306 Gm. and was firm and rubbery. The surface was covered with a mild fibrinous exudate. The cut surface was purplish red, and large amounts of pulp of a similar color could be scraped from it. The follicles were prominent, large and well demarcated. Histologically, there were numerous



Fig. 2.—Liver showing necrosis of vessel walls with the lumen filled with yeast-laden phagocytes. Yeasts englobed by the Kupffer cells are less distinct at this magnification ($\times 100$).

regions of necrosis containing nuclear dust surrounded by numerous macrophages distended with the yeast forms of the organism. Intermingled with the phagocytes were numerous fibroblasts. Between the necrotic regions the splenic structure was obliterated with extensive fibroblastic proliferation and yeast-laden phagocytes. Impression smears from the spleen stained by Wright's and Giemsa's technics showed large numbers of the organisms within the phagocytes.

The right and left kidneys weighed 54 and 57 Gm., respectively. The cut surfaces were pale pink, and the cortical striations were scarcely visible. Scattered throughout the cortices were a few small, light yellowish brown regions suggestive of tubercles. The medullary portions were somewhat darker, and the radial striations were clearly visible, thus sharply demarcating these portions from the cortices. There were no grossly visible abnormalities of the papillae, calices, pelves or ureters. Histologically, the kidneys showed scattered foci of fibroblastic proliferation, especially in the cortices, although these foci were present throughout

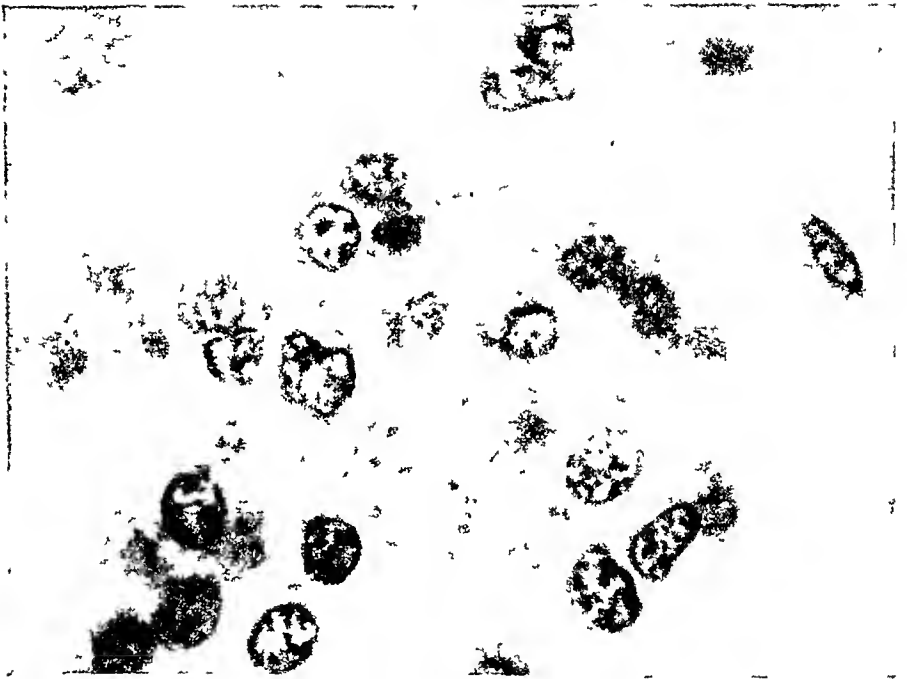


Fig. 3.—Glomerulus showing *Histoplasma capsulatum* in the capillary loops ($\times 1,350$).

the organs. Such regions contained yeast-laden phagocytes and fibroblasts, with minimal leukocytic infiltration. The capsules of Bowman contained some albumin. There was evidence of diffuse proliferation of the glomerular tufts, and in many of the glomeruli, organisms of *Histoplasma* could be seen in groups of three or four (fig. 3). The tubules showed mild granular degeneration. In the mucosa of the renal pelvis were many phagocytes filled with the yeast forms of *Histoplasma*.

The right adrenal gland appeared grossly normal, but there were small regions resembling miliary granuloma in the medulla of the left adrenal gland. The dark cortical portions were sharply demarcated from the medullary portions. The medulla had the appearance of being increased in amount and in some regions extended to the surface by replacing the cortex. There were no grossly visible localized regions of necrosis or hemorrhage. Histologically, the medullae were replaced with macrophages filled with yeastlike forms. There was extensive destruction of the fascicular layer, with the organisms actually existing in the cortical cells. The reaction appeared to be that of a progressive disintegration extending from the medulla outward by contiguous invasion of the cells of the cortical fasciculi (fig. 4 *a* and *b*). In some places this invasion had extended entirely through the cortical layer to the surface of the gland. There was only mild fibroblastic proliferation in the adrenal glands in contrast with that seen in the other organs.

There were no grossly visible lesions in the esophagus, the stomach, the small or the large intestine. However, histologic examination of the esophagus showed focal accumulations of yeast-laden phagocytes with an associated fibroblastic proliferation beneath the mucosa (fig. 5 *a*). The reaction suggested that, if the patient had lived longer, ulceration would have taken place eventually. It also suggested that the ulcer formation cited by other investigators may be due to breaking down of such foci resulting from hematogenous dissemination and not to erosion of the surface from without. In the large bowel there were regions in which the lamina propria was distended with phagocytes containing enormous numbers of *Histoplasma* (fig. 5 *b*). In these regions there was desquamation of the epithelium, forming microscopic ulcers. In many regions the organisms were present in clusters of phagocytes beneath the serosa and between the muscular layers.

The urinary bladder was grossly normal. Microscopically, there were collections of phagocytes only in the outer portion of the wall in the connective tissue. The urine was slightly cloudy but did not have the gross appearance of pus. Microscopic examination showed it to contain phagocytes filled with yeastlike organisms.

There was generalized enlargement of the lymph nodes, which remained discrete. The nodes from the periaortic, pelvic, mesenteric, peripancreatic, cervical, axillary and inguinal regions were similar grossly and microscopically. Histologically, they showed regions of necrosis and fibroblastic proliferation with large numbers of phagocytes filled with *Histoplasma*. Impression smears taken from them and stained by the Wright and Giemsa technics showed enormous numbers of organisms within the phagocytes. There were also many extracellular organisms diffusely scattered throughout the nodes.

The thymus was nodular and pale pink. There were no grossly visible lesions present, and there did not appear to be a significant increase of resistance. Microscopically, there was extensive proliferation of fibroblasts with a few regions of necrosis containing nuclear dust like that seen in caseous tuberculosis. The periphery of such necrotic regions showed fibroblastic proliferation without leukocytic infiltration. Diffusely scattered throughout the tissue were enormous numbers of macrophages engorged with organisms in the yeast form (fig. 6).

A proliferative reaction in tissue adjacent to one parathyroid gland extended into the gland (fig. 7). In the remaining three parathyroid glands there was no microscopic evidence of invasion by, or reaction to, the organisms.

Marrow from the ribs and the vertebral bodies was reddish brown and grossly appeared normal. The impression smears stained by the Wright and Giemsa technics showed enormous numbers of organisms in the phagocytes.



Fig. 4.—(a) Adrenal gland showing replacement of the medulla with myriads of yeast forms in phagocytes, the process extending outward into the cortical fasciculi ($\times 100$). (b) Cortical cells of (a). Note the distention with large numbers of organisms and the obliteration of architecture ($\times 1,350$).

There were no gross or microscopic lesions in the dura, the pia-arachnoid, the cerebrum, the cerebellum, the spinal cord or the pituitary gland. However, a

section of the spinal cord showed organisms within phagocytes in the blood vessels of the meninges, immediately adjacent to the cord. This would be expected, however, in view of the presence of organisms circulating in the blood as demonstrated by bacteriologic technic. The middle ears were normal.

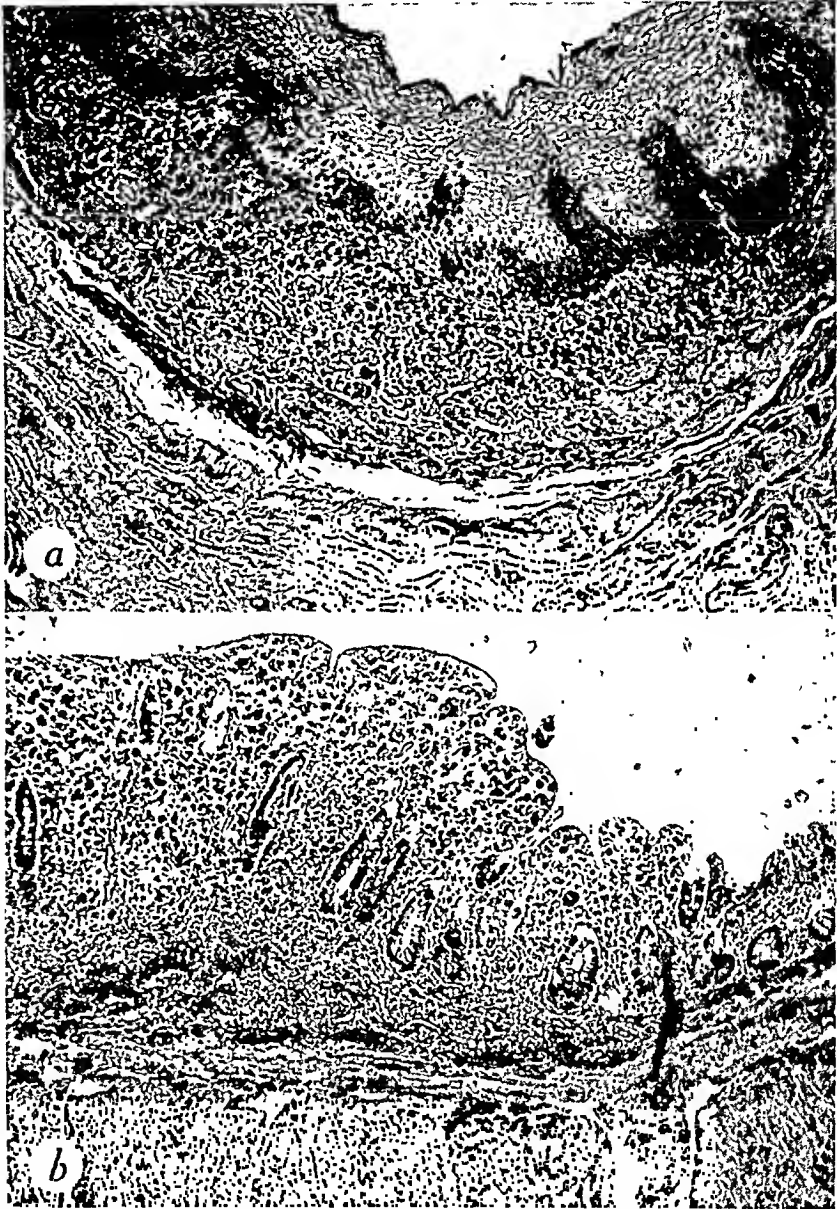


Fig. 5.—(a) Esophagus showing accumulation of yeast-filled phagocytes and fibroblastic proliferation in the submucosa ($\times 75$). (b) Large bowel, showing numerous yeast-filled phagocytes distending the lamina propria ($\times 50$).

The diaphragm was grossly normal, but microscopic examination revealed numerous foci of accumulations of yeast-laden phagocytes intermingled with fibroblasts (fig. 8a).

Bacteriologic Examination.—The following materials were subjected to bacteriologic examination in an attempt to isolate the organism *H. capsulatum*: blood, lung, liver, spleen, kidney, rectus abdominis muscle, lymph nodes, frontal lobe of

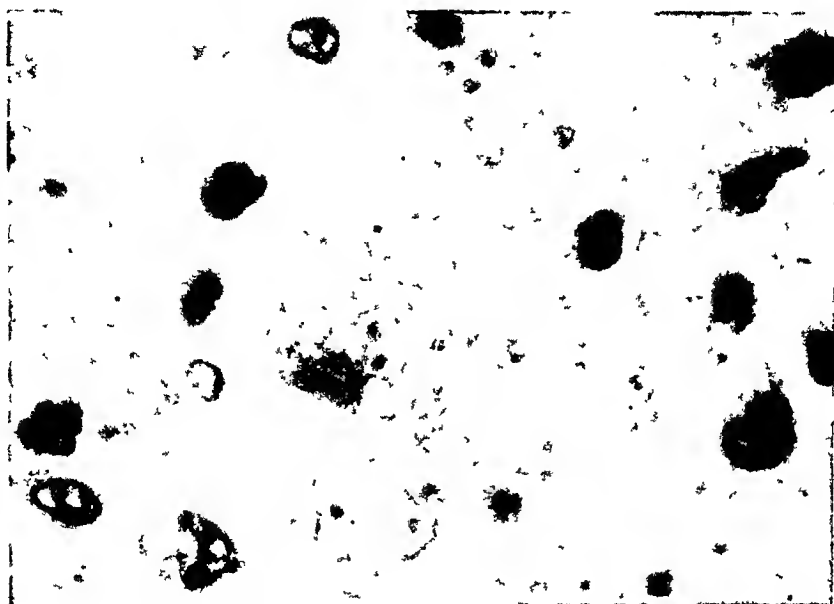


Fig. 6.—Thymus showing organisms in the phagocytes ($\times 1,350$).



Fig. 7.—Parathyroid gland. The fibroblastic proliferation at the left is extending into the gland as dense cords ($\times 85$).

brain, marrow from a vertebral body, cerebrospinal fluid, bile, urine and stool. Blood was drawn aseptically from the heart after the skin over the precordium had been cauterized. Cerebrospinal fluid was removed from the cisterna magna in a similar fashion. Likewise a sample of urine was obtained from the urinary

bladder and bile from the gallbladder. Marrow was obtained from the vertebral bodies by squeezing them and removing the extruded material. Specimens of lung, liver, spleen, kidney, rectus abdominis muscle, lymph nodes and frontal lobe of the brain were removed aseptically by cauterizing the surface of the respective organ and excising the tissue with sterile scissors. These instruments were submitted to dry heat at 170 C. for two hours to eliminate the theoretic possibility that organisms might be carried over on them, as might have been the case had they been prepared by boiling.

Blood, cerebrospinal fluid, urine and bile were cultured directly. The tissues and the stool specimen examined were emulsified in saline solution, and the

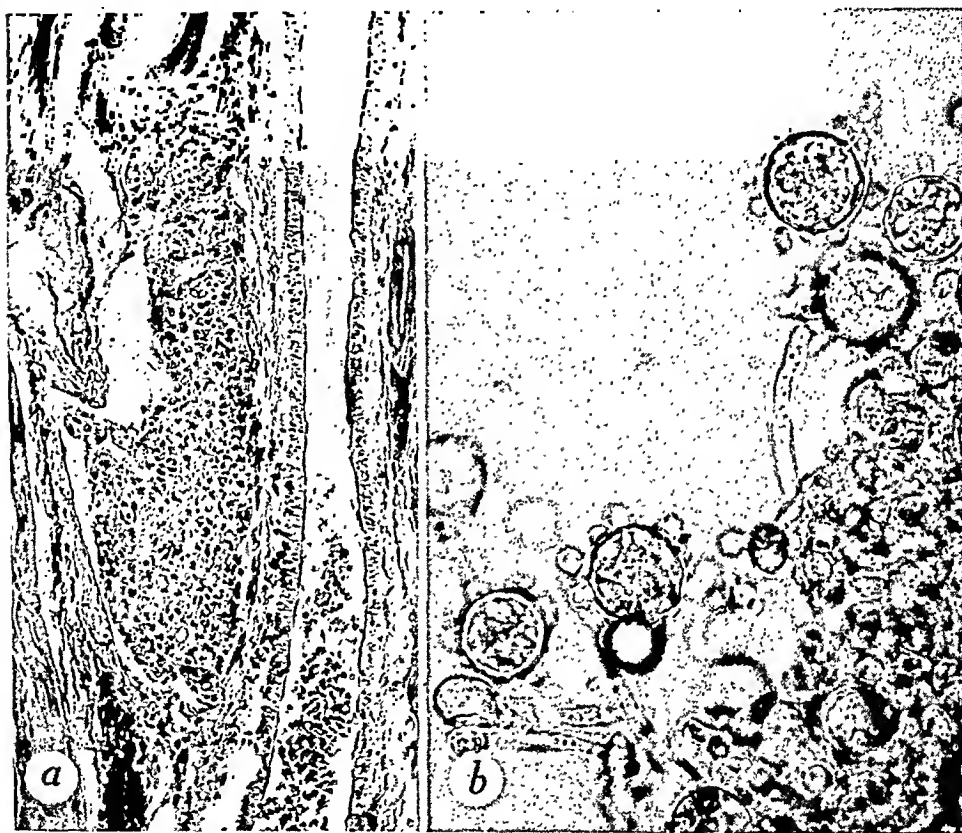


Fig. 8.—(a) Diaphragm showing numerous phagocytes filled with organisms ($\times 105$). (b) Tuberculate chlamydospores produced by the organism isolated from the patient ($\times 900$).

emulsions were used as inoculums. The materials were cultured on 7 per cent horse blood agar containing 20 units per cubic centimeter each of penicillin and streptomycin, and the plates were incubated at 30 to 32 C. and at 38 C. Portions of blood, cerebrospinal fluid, bile, urine and emulsions of spleen, liver and lung were also inoculated in 7 per cent horse blood broth at 38 C. in an attempt to produce cultures in the yeast form.

Growth was more rapid in plates incubated at 30 to 32 C. than in those incubated at 38 C. No yeast forms were produced at the higher temperature, although filamentous forms were numerous. At 32 C. numerous tiny white microaerophilic filamentous colonies were present after four to five days. These gradually increased

in size, ranging from 5.0 to 20.0 mm. in diameter, depending on the crowding of the colonies. When the inoculum was heavy—for example, those from the spleen, lymph nodes or marrow—the adjoining colonies fused to form a matlike growth. The early growth was composed only of branching filaments radiating from a central tangled mass. Colonies seven to ten days old contained well developed chlamydospores. In colonies two to three weeks of age the diagnostic tuberculate chlamydospores developed as seen in figure 8*b*. The organisms were isolated from specimens of blood, lung, liver, spleen, kidney, marrow, rectus abdominis muscle, frontal lobe of the brain, urine and stool. They were not isolated from cerebrospinal fluid or bile. The absence of the organisms from the cerebrospinal fluid and the lack of histologic evidence of involvement of the brain suggest that the cultural demonstration of organisms in the frontal lobe was probably due to their presence in the blood vessels supplying the tissue examined. After the material had been kept for six weeks in the icebox, the organisms were still viable and retained their original characteristics.

COMMENT AND SUMMARY

The evidence obtained from a study of the pathologic material of this case indicates widespread dissemination of the *Histoplasma*. The finding of the organisms in the blood, the marrow and lymph nodes suggests that every organ and tissue was involved to some degree, although on microscopic examination some specimens showed no fixed tissue reaction to the presence of the organisms. Whether this represents variation in tissue immunity or some other mechanism cannot be determined from the information at hand. The condition of the adrenal glands is especially interesting in this connection. The yeast forms appeared to be actually invading the cortical cells, some of the latter containing so many parasites that they lost their identifying characteristics and appeared as macrophages. To consider either the cortical cells as phagocytes or the yeast forms as invaders is unique since in no other condition has the former been demonstrated and in other parts of the body the organisms occurred only in phagocytic cells.

CONGENITAL TULAREMIA

THOMAS N. LIDE, M.D., DURHAM, N. C.

REPORTS of tularemia occurring during pregnancy are not numerous and are most often included in general considerations of the disease. None I have been able to find mentions either tularemic interruption of pregnancy or tularemia occurring in the fetus. Bowe and Wakeman¹ recorded a case in which the mother acquired tularemia during midpregnancy, recovered and delivered a normal infant whose blood agglutinated *Pasteurella tularensis* in dilutions up to 1:80 at the time of birth. Three other patients are mentioned by Kavanaugh.² All went to term and delivered normal infants. Pullen and Stuart³ likewise mentioned 3 patients who acquired the disease during pregnancy and later delivered apparently normal infants.

The present case of fetal infection with intrauterine death, and recovery of the mother is thought to be the first reported.

REPORT OF A CASE

A 30 year old white married woman, octipara and in the eighth month of her ninth pregnancy, was admitted to Duke Hospital on Dec. 3, 1945, complaining of a "sore" on the left third finger of about two weeks' duration. She had prepared a rabbit, caught by a dog, one week before the onset of her illness, and had subsequently during that week dressed or prepared two other rabbits for the table. A small ulcer appeared on the left third finger, associated with enlargement and pain of the epitrochlear and axillary lymph nodes and general aching. One week after the onset, nausea and vomiting developed after meals, and she noted that her temperature was elevated, with daily fluctuations rising as high as 39.4 C. (102.9 F.). She had no chills. More exact relationships of exposure and development of symptoms were not remembered.

Her last menstrual period was in April 1945, and the expected date of confinement was in January 1946.

It is of interest that two sons of the patient, aged 14 and 8 years, were confined to this hospital at the same time as the mother. Both had tularemia. In the older boy the disease was ulceroglandular, while in the younger it was of the oculo-glandular type. Both recovered.

Physical Examination.—The patient's temperature was 37.5 C. (99.5 F.); pulse rate, 100; respirations, 18. The patient appeared chronically ill, but was a well developed and well nourished white woman. There was an ulcer 1.5 cm. in diameter with a necrotic base on her left third finger. The left epitrochlear and axillary lymph nodes were enlarged and tender. No other cutaneous or lymphatic

From the Department of Pathology, Duke University School of Medicine.

1. Bowe, D. P., and Wakeman, D. C.: J. A. M. A. **107**:577, 1936.

2. Kavanaugh, C. N.: Arch. Int. Med. **55**:61, 1935

3. Pullen, R. L., and Stuart, B. M.: J. A. M. A. **129**:495, 1945.

lesions were noted. The heart and the lungs were clear. The fundus of the uterus was palpable 2 cm. above the umbilicus; fetal movements were active, and fetal heart sounds were heard best in the right lower quadrant of the abdomen.

Accessory Clinical Findings.—At the time of admission the leukocyte count was 7,800 per cubic millimeter, with a differential count of 58 per cent segmented, 15 per cent stab and 5 per cent juvenile neutrophilic polymorphonuclear leukocytes and 22 per cent lymphocytes. The corrected sedimentation rate was 36 mm. per hour. An uncatheterized specimen of the urine contained 3 to 10 red blood cells per high power field but was otherwise normal. Agglutination of *P. tularensis* occurred in dilutions of 1:2,560 on the day of admission.

Course in Hospital.—The patient was afebrile for the first seven days of her hospital stay. The ulcer on the finger healed well under treatment with saline compresses. Agglutination tests for *P. tularensis* on the fourth and eighth days of admission resulted in the same agglutination titer as did that of the day of admission. On the sixth day the patient stated that she felt no fetal movements, but the fetal heart sounds were still audible in the right lower quadrant of the abdomen. On the eighth day her temperature rose to 38.6 C. (101.4 F.) and on the ninth day to 40.5 C. (104.9 F.). Her leukocyte count had meanwhile risen to 15,200 per cubic millimeter, with a differential count of 51 per cent segmented, 39 per cent stab and 5 per cent juvenile neutrophilic polymorphonuclear leukocytes and 5 per cent lymphocytes. Fetal heart sounds were no longer heard. On the tenth day the patient began to discharge a bloody material per vaginam. On the eleventh hospital day she delivered a macerated male fetus, after which her temperature fell to normal, with subsequent daily fluctuations no higher than 37.8 C. (100 F.). The leukocyte count also fell to normal and remained between 5,200 and 8,900 per cubic millimeter, with normal differential counts. Subsequently her illness was complicated by jaundice, hepatomegaly and splenomegaly, which began on the third postpartum day and cleared rapidly, so that she was discharged to her home on the twenty-second hospital day (eleven days post partum).

Blood taken from the umbilical cord at the time of delivery of the fetus agglutinated *P. tularensis* in dilutions of 1:2,560.

Autopsy of the Fetus (two days after delivery and approximately five days after death).—The macerated male infant was well formed, measuring 46 cm. in crown-heel length and weighing 2.4 Kg. Sanguineous fluid was found in all the body cavities, and there was gross evidence of postmortem degeneration of all organs.

The heart weighed 19 Gm. and showed no abnormalities. The lungs showed no gross lesions. The spleen weighed 16 Gm. It was firm, enlarged and dark red. The pulp bulged slightly above the cut surface of the capsule. The liver weighed 107 Gm. (normal for this age, approximately 70 Gm.). It was dark red without evidence of localized inflammatory changes. The adrenal glands showed no gross lesions. The weight of the kidneys combined was 23 Gm. These organs were darker than usual and showed only slight fetal lobulations. The thymus weighed 11 Gm. and showed no gross lesions. The brain was so badly autolyzed that it could not be studied. The placenta measured 17 by 12 by 5 cm. and weighed 450 Gm. No gross lesions were noted.

Microscopic Examination.—Throughout the placenta were many granulomatous foci, located predominantly in the intervillous spaces. These foci were composed of round cells, both lymphocytes and mononuclear plagiocytes, and a few polymorphonuclear leukocytes enmeshed in deposits of fibrin. Karyorrhectic necrosis

at the center of these lesions was a prominent feature. Many lesions were confluent and involved adjacent chorionic villi, which themselves contained many interstitial mononuclear cells, with necrosis in some of the villi. It appeared that the cells from the fetal circulation were taking an active part in the inflammatory reaction.

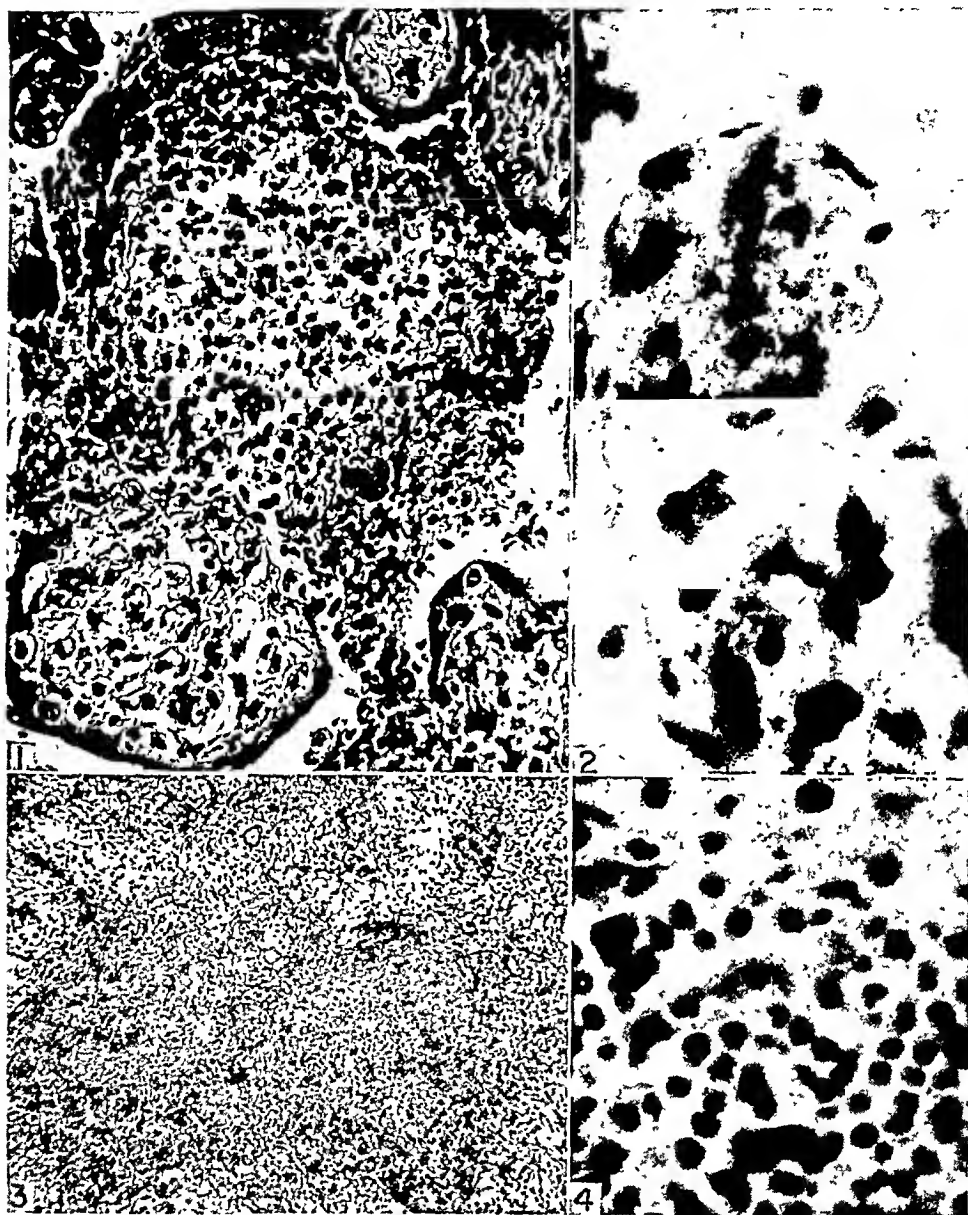


Fig. 1.—Lesion of the placenta. It is characteristic of those found throughout the placenta and shows that chorionic villi were involved in the inflammatory process. $\times 295.5$.

Fig. 2.—Two chorionic villi containing clumps of gram-negative coccobacilli. $\times 1,478$.

Fig. 3.—Lesions of the spleen were similar to those found in the liver, the kidney, the lungs and the marrow. The borders of two additional lesions are visible at the upper margin of the photograph. $\times 73.9$.

Fig. 4.—The mononuclear reaction at the border of the necrotic focus in the capsule of the thymus. $\times 887$.

No lesions were found in the heart.

The capsule of the thymus contained one well preserved lesion, the center of which showed considerable necrosis with surrounding infiltration of round cells and deposition of fibrin. No lesions were noted in the gland itself, which was much better preserved than other tissues.

One small necrotic focus similar to the other lesions was found in the lungs. The tissue was poorly preserved.

The spleen and the liver, despite the marked autolytic changes, showed large numbers of small necrotic granulomas. They were marked particularly by deposits of fibrin with central necrosis and with peripheral infiltration of macrophages and lymphocytes. Many small lesions were confluent.

Similar foci were found in the adrenal glands, in the cortex of each kidney and in the costal marrow.

In sections of the placenta, stained for bacteria by the Brown-Brenn⁴ technique, masses of tiny gram-negative coccobacilli were found within two of the villi. The organisms were consistent morphologically with *P. tularensis*. They are shown in figure 2. Other small gram-negative bodies, apparently the organism, were found also within the macrophages in the placenta and spleen, but no other easily identified masses of bacteria were found.

COMMENT

It is difficult to state with any degree of certainty the time at which the fetus became infected, but evidence suggests that the infection was active in the infant during the fourth week of the mother's illness, when on her sixth hospital day fetal movements became less notable, with fetal death occurring on the ninth day. The mother had been hospitalized on about the twentieth day of her illness. The rise of temperature and of leukocyte count which occurred on the eighth hospital day may well represent her reinfection by the fetus. This suggestion is supported by the fact that the organisms were found within chorionic villi, with placental necrosis, and by the direct communication between maternal and fetal circulations. The fact that the temperature and the leukocyte count rapidly returned to normal after delivery also supports the suggestion of reciprocal infection.

The finding of the organisms in the tissues is also unusual. Lillie and others⁵ have pointed out the difficulty of identifying *P. tularensis* in human material, and in only 2 of the 26 cases which they reviewed was the organism found in the tissues. In 3 cases reported by Thomas⁶ organisms consistent morphologically with *P. tularensis* were found in smears of material taken from various organs at autopsy. Matthews⁷ (cited by Ashburn and Miller) reported finding the organisms in tissues taken at autopsy in 1 case. Ashburn and Miller⁸ also found intra-

4. Brown, J. H., and Brenn, L.: *Bull. Johns Hopkins Hosp.* 48:69, 1931.

5. Lillie, R. D., and others: *The Pathology of Tularaemia*, National Institute of Health Bulletin 167, United States Treasury Department, Public Health Service, 1936, pp. 73-75.

6. Thomas, H. B.: *Ann. Int. Med.* 17:659, 1942.

7. Matthews, W. R.: *New Orleans M. & S. J.* 90:479, 1938.

8. Ashburn, L. L., and Miller, S. E.: *Arch. Path.* 39:388, 1945.

cellular organisms, which they identified as *P. tularensis*, within macrophages in the lung of a patient who died during the fifth day of her illness.

SUMMARY

A case of congenital tularemia, presumably the first, is presented. The disease occurred in the mother during the eighth month of pregnancy and was followed by infection and death of the fetus. The necrotic granulomas occurring in various organs of the fetus were similar to those which are usually found in adults. Gram-negative coccobacilli consistent morphologically with *P. tularensis* were demonstrated in chorionic villi.

AINHUM

Report of a Case in Which the Patient Was a White Woman with Diabetes Mellitus

L. J. ORDIE SHAFFER, M.D., MINNEAPOLIS

AINHUM (dactylolysis spontanea) has been differently considered by various authorities as being either a distinct pathologic entity or a symptomatic manifestation of any one of various diseases. The condition is characterized by a gradually constricting fibrous ring most commonly in the digitopltar fold of the little toe, which deepens and eventually results in a spontaneous amputation. The condition is most commonly found in male Negroes and is more prevalent in Africa, South and Central America and the West Indies but variations in the incidence as regards sex, race and toe involved occur, and 51 cases have been reported in the United States. Clark first noted this condition among the natives of the African Gold Coast in 1860 and reported it under the name of "dry gangrene of the little toe." In 1867 da Silva Lima¹ gave the first adequate description of the condition. Hornaday² published the first report of a case in the United States in 1881. Since that time arguments have been advanced for and against the view that ainhum is a distinct disease and although the majority favor the idea that it is an entity, there is evidence supporting the other view. The present report of a case seems timely as it gives added weight to the view that ainhum does occur as a manifestation of various local and general pathologic conditions.

REPORT OF CASE

A white woman aged 57 was known to have been diabetic for six years and had taken 20 units of crystalline insulin daily during the year 1945. She first noticed a corn on the lateral side of the left third toe in July 1944. A minimal infection of the skin developed in the area and continued for two months. At the end of this time all infection had subsided, but a constricting band was present around the toe. The constriction continued to progress during the next sixteen months until the globular toe was attached only by a narrow pedicle. No color changes or further infection occurred in the third toe during the intervening sixteen months. During October 1945 a corn developed on the left fourth toe, and a similar constriction on the lateral and inferior surface of the toe occurred during the following two months. The patient had noted cramps in her legs on walking since August 1945, indicative of intermittent claudication. In December 1944 she bumped her left great toe and a mild infection of the skin occurred in the abrasion. This healed shortly thereafter, but in July 1945 an infection recurred in the great toe, and small amounts of purulent material drained from

From the Department of Surgery, University of Minnesota.

1. da Silva Lima, J. F.: *Arch. Dermat.* 6:367, 1880.

2. Hornaday, E. H.: *North Carolina M. J.* 8:116, 1881.

the area periodically from that time on. In October 1945 the great toe became painful, and the following month a black discoloration of the medial portion of the great toe occurred. The history of the patient revealed that she was born in Canada of Irish descent but had lived in Minnesota during the last thirty-five years. She had had none of the usual childhood diseases except measles. At 31 years of age a subtotal hysterectomy had been done for uterine myoma. She said that she had never had a venereal disease. One sister also had diabetes, but no other member of the family had any similar disturbance of the feet. On admission to the University Hospital, December 31, she was a moderately obese, well developed white woman in no acute distress. There was diabetic retinopathy with increased tortuosity of the vessels and scattered "cotton wool" exudates. No hemorrhages or papilledema were present however. The mouth was edentulous but otherwise normal. The blood pressure was 140 systolic and 80 diastolic. Neck, heart, lungs

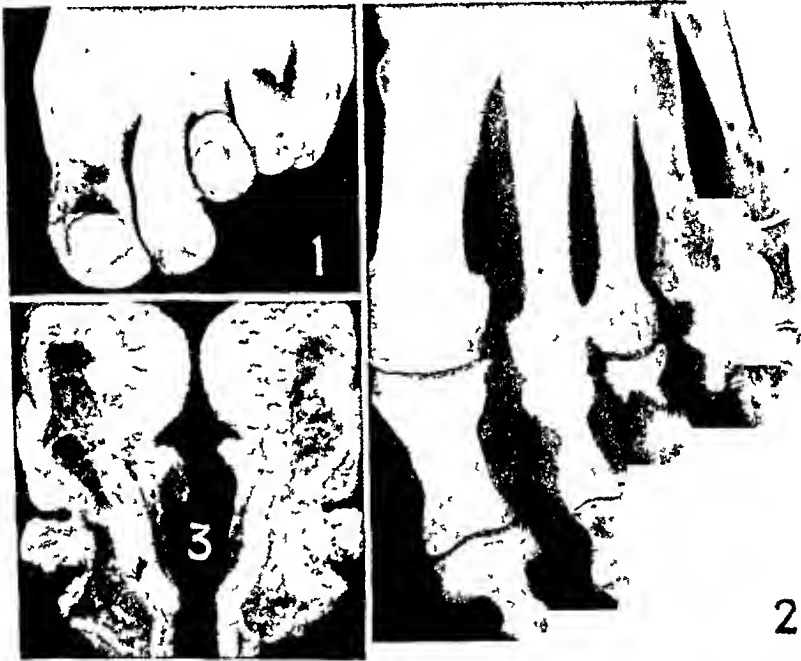


Fig. 1.—Photograph of the left foot showing ainhum of the third toe, beginning constriction of the fourth toe and local areas of superficial dry gangrene on the dorsum of the hallux and the lateral side of the foot.

Fig. 2.—Roentgenogram of the left foot revealing complete severance of the third proximal phalanx and beginning atrophic changes in the fourth proximal and middle phalanges.

Fig. 3.—Medial and lateral halves of the longitudinally sectioned third left toe involved by ainhum. Note the fibrous band severing the proximal phalanx.

and abdomen were all normal except for the presence of a small midline infra-umbilical incisional hernia. There was no perceptible arterial pulsation in either foot, but the right foot was otherwise normal. A deep circular constriction was present around the midportion of the proximal phalanx of the left third toe, which had almost severed the toe. No signs of local inflammation or tenderness were present, however, and the toe could be moved in any direction much as a grape on a stem. A corn was present on the lateral dorsum of the fourth left toe with a constriction on the lateral and inferior surfaces. Areas of dry gangrene without infection were present on a 2 by 5 cm surface of the medial hallux and on a

1 by 1 cm. area on the lateral surface of the foot at the distal end of the fifth metatarsal. No swelling or erythema of the foot was present (fig. 1). The urine had a specific gravity of 1.025; the hydrogen ion concentration was 5; there was a trace of albumin, sugar (1 plus) and acetone (1 plus); there was no diacetic acid; there were no casts or erythrocytes; the leukocyte content was 3 plus. The hemoglobin value was 12.7 Gm.; the blood leukocyte count was 8,300, with neutrophils 79 per cent and lymphocytes 21 per cent. The sedimentation rate was 91 mm. in one hour (Westergren method). The Kline and Kahn tests were negative. The blood sugar was determined to be 181 mg. per hundred cubic centimeters; the carbon dioxide-combining power, 44 volumes per cent; the blood urea nitrogen, 8 mg. per hundred cubic centimeters and the plasma proteins, 6.4 Gm. per hundred cubic centimeters. Culture of the urine revealed *Acrobacter*, and culture of the surface of the area of dry gangrene revealed only coagulase-negative staphylococci, nonhemolytic streptococci and diphtheroids present.

Röntgen examination of the left foot revealed a constriction of the soft tissue through the middle of the proximal phalanx of the third left toe with rarefaction and complete severance through the center. Atrophic changes were present in the third middle phalanx, and only a very small portion of the third distal phalanx was visible. Atrophic changes were also visible in the proximal and middle phalanges and the intervening joint space of the fourth toe (fig. 2). The changes were compatible with either ainhum or leprosy, but diagnosis of the latter was not favored, since there were no other bacteriologic or physical findings to substantiate it.

The patient was given parenteral penicillin therapy together with foot soaks for eleven days in the hope that these measures might aid in the demarcation and slough of the superficial dry gangrene. However, no improvement occurred. The first four toes were then amputated and a primary closure done in each case. Unfortunately, as was suspected, although no infection occurred, healing was impaired because of vascular disease. Three weeks were given to offer every possible chance of healing, and then, after McClure-Aldrich tests had revealed impairment below the knee, and histamine flare tests an impairment below the middle of the lower leg, a supracondylar amputation was done on the left leg. The stump healed by primary intention, and the patient was discharged from the hospital on the tenth postoperative day, Feb. 18, 1946. She has had no further difficulty in the short interval since that time.

Pathologic Examination.—Gross examination of a longitudinal midsection of the third left toe revealed a fibrous constriction that had completely severed the bone (fig. 3). A beginning constriction was present on the lateral and inferior surface of the fourth toe. The second toe was normal, but the great toe showed an area of dry gangrene 2.5 by 5 cm. in size on the medial surface. Microscopic examination of the tissue of the third toe revealed the epidermis to be hypertrophied at the point of constriction and just distal to this area. The stratum corneum was moderately thickened. A poorly defined stratum lucidum was present, but the stratum granulosum was normally developed. The stratum malpighii (stratum germinativum) was markedly thickened with elongated, enlarged dermal papillae present, and the plexiform rete cones freely anastomosed with one another. The normal nuclear structure was present in both the stratum granulosum and the stratum malpighii. The reticular layer of the dermis was composed of bundles of collagenous fibers, but a moderate increase in the amount of elastic fibers was present throughout the dermis. There was a slight perivascular infiltration of round cells. The small arteries showed no change except slight intimal thickening, and

the medium-sized arteries showed slight medial degeneration, but both changes were within the normal limits of change due to age. The veins were entirely normal. Simple atrophy of bone was present, with narrowing of the trabeculae and reduction of osteoclasts. The areas between the irregular trabeculae of the third proximal phalanx were replaced by dense collagenous connective tissue at the point of the severed bone, and beginning constriction was present on the proximal two phalanges of the fourth toe. The nerves of the toe had a normal structure.

Examination of the amputated leg revealed a necrotic ulcer 2.5 cm. in diameter over the lateral malleolus and a 2 cm. ulcer over the fifth tarsometatarsal joint. The sites of amputation of the first four toes showed incomplete healing. Dissection of the vessels showed the popliteal artery to be moderately sclerotic without appreciable narrowing. The anterior tibial artery was sclerotic and 60 per cent narrowed. The orifice of the peroneal artery was narrowed. The posterior tibial artery was occluded by a hyaline thrombus 4 cm. distal to the bifurcation. Microscopic sections revealed an atheromatous plaque in the popliteal artery causing a small amount of narrowing. No calcification was present in the wall. Section of the anterior tibial artery showed sclerosis of the wall with medial ossification. Section of the posterior tibial artery showed a hyaline thrombus which was partially canalized. The findings were therefore those of arteriosclerosis with arterial thrombosis in the larger vessels of the leg, but the blood vessels of the third and fourth toes were within normal limits of age change for the patient. Thus vascular changes alone would not account for the developing spontaneous amputations. Hyperkeratosis and parakeratosis were present in the epidermis at the site of the constriction of the toes, and contracting collagenous fibers had completely severed the proximal phalangeal bone of the third toe and were constricting the proximal phalangeal bones of the fourth toe, resulting in the clinical manifestation of ainhum.

ETIOLOGY OF AINHUM

Numerous theories have been advanced as to the cause of ainhum but none is satisfactory. It seems reasonable to consider ainhum as a manifestation of various diseases rather than a separate disease.

Local Diseases of the Skin.—Spinzig³ noted that in 8 of the reported cases of ainhum in the United States corns or calluses were localized at the point of constriction. Unna has been cited as considering ainhum a local ring form scleroderma causing endarteritis and rarefying osteitis. Despetits and Corre⁴ and da Silva Lima¹ also favored this idea, and Grschebin⁵ reported Barthelémy, Besmer and Leistrkow as considering ainhum to be on a basis of local scleroderma. In Snider's⁶ case, presented in 1929, there was not complete agreement as to the diagnosis of ainhum, and the possibility of the condition having been caused by a fungous infection was mentioned. In reference to this case Tobias commented that "ainhum includes numerous diseases of the small toes." Later that same year Gross⁷ presented a case of ainhum which he believed due to trauma and trophoneurosis.

3. Spinzig, E. W.: Am. J. Roentgenol. **42**:246, 1939.

4. Scheube, B.: Die Krankheiten der warmen Länder, Jena, Gustav Fischer, 1900, p. 632.

5. Grschebin, S.: Urol. & Cutan. Rev. **40**:98, 1936.

6. Snider: Arch. Dermat. & Syph. **20**:139, 1929.

7. Gross: Arch. Dermat. & Syph. **21**:874, 1930.

Nonlocalized Diseases of the Skin.—The occurrence of ainhum has been reported several times associated with widespread disease of the skin. Hyde and Montgomery⁸ in 1904 referred to 3 white patients with palmar and plantar keratoses in whom ainhum had also developed. They considered ainhum as scleroderma annulare "originat... the causes found effective in the ordinary types of scleroderma." Pavlovskoi and Karishevoi⁹ reported a case in which ainhum developed in a 17 year old Russian girl with palmar and plantar keratoses, and Wigley,¹⁰ a case of ainhum-like constriction of the fingers in a 10 year old white girl with palmar and plantar keratoses. Grschebin⁵ cited Pardo-Castello and Mestra, who saw 6 patients with ainhum over a fifteen year period and expressed the opinion that ainhum can be caused by various conditions. One of their patients had leprosy and another keratoderma. The latter patient came from a family in which 7 members had keratoderma, and in 3 of these this had resulted in ainhum. Grschebin⁵ reported the case of an 18 year old Russian girl with general ichthyosis and keratosis palmaris et plantaris in whom ainhum of both little toes developed. He also cited Pavlowskaja-Karyschewa, who reported a case in which ainhum occurred in a 12 year old Russian girl six years after the development of scleroderma, and Pospelow, who described a patient with sclerodactylia in whom ainhum developed. Stelwagon¹² observed ainhum in a 28 year old man with a cutaneous condition thought to be pityriasis rubra pilaris; there had been loss of one small toe, and there were beginning constrictions of other toes and one little finger. The development of ainhum in these varied dermatologic conditions adds further support to the view that ainhum is a symptom and not a disease.

Injury and Mechanical Irritation.—Heitzmann¹³ favored the idea of self-induced trauma as the cause of ainhum since he had observed cases in which local ligatures or strings were important etiologic factors. Eyles¹⁴ favored the theory of an injury of the digitoplantar fold in which there was local introduction of foreign material causing hyperplasia of the epidermis, with subsequent pressure on the vasomotor nerves producing the trophic phenomena. Manson¹⁵ also favored the theory of injury and irritating foreign matter. In addition to the unguarded lateral position of the fifth toe and the greater likelihood of trauma Paterson¹ thought the obliquity of the fourth and fifth flexor tendons of Negroes might be a factor in the traumatic origin of ainhum.

8. Hyde, J. N., and Montgomery, F. H.: *Ainhum*, in *A Practical Treatise on Diseases of the Skin*, ed. 4, Philadelphia, Lea Brothers & Co., 1897, p. 598; ed. 7, Philadelphia, Lea & Febiger, 1904, p. 608.

9. Pavlovskoi and Karishevoi: Abstracted, *J. Cutan. Dis.* **36**:133, 1918.

10. Wigley, J. E. M.: *Brit. J. Dermat.* **41**:188, 1929.

11. Footnote deleted by the author.

12. Stelwagon, H. W.: *A Treatise on Diseases of the Skin*, ed. 8, revised, Philadelphia, W. B. Saunders Company, 1918, p. 656.

13. Heitzmann, C.: *Tr. Am. Dermat. A.* **5**:49, 1881.

14. Eyles, C. H.: *Lancet* **2**:576, 1886.

15. Manson, P.: *Tropical Diseases*, ed. 1, revised, London, Cassell & Co., 1903, p. 725.

Infection.—Shepherd¹⁶ expressed the belief that ainhum is on a basis of local infection, and in 1887 he predicted that an "ainhum bacillus" would be found, but this concept of a specific etiologic organism never proved true. In the same year Horowitz¹⁷ reported a case in which he concluded that infection was the important factor. Wellman¹⁸ expressed the belief that infestation of the skin by *Sarcopsylla penetrans* with associated infection had caused the ainhum in his cases, and Babler¹⁹ and Castellani and Chalmers²⁰ also favored a parasitic origin of the condition.

General Diseases.—Leprosy and syphilis have both been reported as having caused ainhum. Grschebin⁵ in 1922 noted ainhum in 2 patients in the Astrakhan leprosarium. Zambaco-Pacha and Pardo-Castello and Mestre are cited as having also made the same clinical observation. Other workers have noted that leprosy more frequently produces a different clinical picture since anesthesia of the foot permits severe injuries to occur, with ulcers that extend into the joints of several of the toes.

Bharucha²¹ observed ainhum in a Hindu with syphilis and Wright²² in a man with syphilis. Both investigators expressed the belief that syphilis caused the ainhum.

Metabolic Disturbances.—Numerous investigators have seen ainhum which they believed to be on either a neurotrophic or a vascular basis. Shepherd¹⁶ expressed the opinion that a trophic disturbance of the nerve centers had caused ainhum in a patient he had seen. Later other investigators, including Sheube,⁴ Matas,²³ Abbe,²⁴ Pusey²⁵ and Stelwagon,¹² considered a neurotrophic disorder to be the cause of ainhum. Weinstein²⁶ reported that he had found the sensation of the affected toes diminished or increased but never entirely lost. Sutton²⁷ described a child with ainhum who died of an obscure nervous disorder; at autopsy degenerated areas in the cord and peripheral neuritis were observed. Welch²⁸ found the tactile sense slightly impaired in the distal part of the toe, although the reflexes were normal. He expressed the belief that ainhum is due to trophoneurosis plus a traumatic element.

16. Shepherd, F. J.: Am. J. M. Sc. **93**:137, 1887.

17. Horowitz, O.: Med. & Surg. Reporter **56**:649, 1887.

18. Wellman, F. C.: J. Trop. Med. **11**:117, 1908.

19. Babler, E. A.: Ann. Surg. **48**:110, 1908.

20. Castellani, A., and Chalmers, A. J.: Manual of Tropical Medicine, New York, William Wood & Company, 1910, p. 1148.

21. Bharucha, E. S.: Indian M. Gaz. **52**:403, 1917.

22. Wright, L. T.: Urol. & Cutan. Rev. **28**:135, 1924.

23. Matas, R.: Tr. Am. S. A. **14**:483, 1896.

24. Abbe, T.: M. Rec. **79**:478, 1911.

25. Pusey, W. A.: Principles and Practice of Dermatology, ed. 3, D. Appleton and Company, 1917.

26. Weinstein, H.: Proc. Canal Zone M. A. **4**:110, 1911.

27. Sutton, R. L.: Diseases of the Skin, ed. 9, revised, St. Louis, C. V. Mosby Company, 1935, p. 624.

28. Welch, R. S. G.: U. S. Nav. M. Bull. **21**:352, 1924.

Guimares²⁹ expressed the opinion that the cause of ainhum is a contracture of arteries that nourish the toes. This subsequently results in a circulatory deficiency and spontaneous amputation of the toes. According to Ashley-Emile,³⁰ strain on the obliquely placed fourth and fifth flexor tendons produces atrophy of the nerve followed by degeneration of the muscle and rotation of the toe, which produces strangulation of the blood vessels. In the present reported case, there was a vascular impairment of the extremity, but a study of the arteries of the blood vessels near the area of ainhum is convincing that the initial corns and irritation were probably important etiologically. The absence of this phenomenon in the presence of the high incidence of gangrene of the extremities is further evidence of this. No case similar to the present reported case could be found among published instances of gangrene of the extremities.

Doubt regarding the occurrence of ainhum as an independent disease has been expressed by the previously cited authors, who have believed that in their reported cases the lesion was a manifestation of one or another of various diseases. Thus ainhum may be due to a local ring form scleroderma or associated with the pathologic conditions that arise with corns, calluses, fungous infections, palmar and plantar keratosis, keratoderma, ichthyosis, scleroderma, pityriasis rubra pilaris, leprosy, syphilis or neurotrophic or vascular disorders. When no specific disease is demonstrable, the ainhum probably results from scarring and contracture in the digitoplantar fold as a result of trauma and infection. The fibrogenetic character of the Negro further augments this condition and explains the greater incidence in members of that race. Grschebin expressed this view well when he stated, "We support this standpoint, that ainhum is a symptom and not an independent disease and that it may be observed in a number of diseases with similar pathogenesis." More recently Ash and Spitz³¹ have stated that "ainhum is probably not a specific entity but a complication that may arise." The present case supports this view and adds additional evidence that ainhum is a symptom rather than an independent disease.

SUMMARY

The clinical picture of ainhum with a toe being spontaneously amputated by a constricting fibrous ring at its base may occur as a symptomatic manifestation of many local or general diseases. A case is presented in which ainhum occurred in a white patient with diabetes mellitus, affording further evidence that ainhum is a symptom rather than a distinct disease.

29. Dell'Orto, J.: *New Orleans M. & S. J.* 8:516, 1880-1881.

30. Ashley-Emile, L. E.: *J. Trop. Med.* 8:33, 1905.

31. Ash, J. E., and Spitz, S.: *Ainhum*, in *Pathology of Tropical Diseases*, Philadelphia, W. B. Saunders Company, 1945, p. 344.

General Reviews

METASTATIC CALCIFICATION

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THE PHENOMENON of metastatic calcification has been known for nearly a century.¹ A comprehensive modern review of the subject has not been found. To the earlier literature Askanazy² contributed an analysis of the human cases reported up to 1901, to which he added 2 cases of his own. He particularly emphasized bone disease, neoplastic and otherwise, as an underlying cause of metastatic calcification and pointed out the association of renal disease in some of these cases. Since that time chronic renal disease, primary neoplasms of the parathyroid glands and hypervitaminosis D have been demonstrated to cause metastatic calcification in man. Some human cases with no definitely proved etiologic basis have been recorded. Experimentally, vitamin D, parathyroid extract and mineral diets have been employed to produce metastatic calcification in animals. The main purposes of this review are to analyze the reported human cases in which anatomic findings were recorded, to correlate significant clinical details when possible, to summarize available experimental evidence, especially with regard to studies of tissues, and to elaborate the mechanisms involved in metastatic calcification.

Before attempting such a review, one should formulate some definition of the term "metastatic calcification." Virchow¹ thought that the calcium deposits seen in the lungs and the stomach in his cases represented direct calcification of the tissue by which lime salts of the blood penetrated and filled up the tissue. Wells³ stated that in this condition calcium salts are deposited throughout the body in apparently perfectly normal tissues, but especially in the lungs, the kidneys and the gastric mucosa, sites where excretion of acid causes a more alkaline reaction in the tissues concerned and results in the precipitation of calcium salts, less soluble because of the lowered concentration of hydrogen ions in these tissues. In other words, metastatic calcification is a condition in which calcium

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1. Virchow, R.: *Virchows Arch. f. path. Anat.* 8:103, 1855.

2. Askanazy, M., in *Festschrift für Max Jaffe*, Braunschweig, F. Vieweg & Sohn, 1901, pp. 208-240.

3. Wells, H. G.: *Arch. Int. Med.* 11:721, 1911.

salts are deposited in tissues previously normal but with a tendency toward alkalinity.

METASTATIC CALCIFICATION AND BONE DISEASE

The original case of metastatic calcification was that described by Virchow.¹ The patient was a young girl who suffered from rheumatism, loss of weight and bone pain. Cancerous nodules were found in the skeletal bones, chiefly in the vertebrae and the skull; calcium deposits were present in the lungs and the stomach, and renal calculi were found. His second case¹ will be described in the next section of this review. His third case¹ was that of a 15 year old girl who had two recurrences of a myxosarcoma of the left cheek during three months. Metastatic nodules were observed in the skull bones, the brain, the lungs and the intestine and calcium deposits in the lung. His fourth patient¹ was a youth 19 years old, who had been afflicted for sixteen years with necrosis of the proximal end of the left femur. Operation for removal of a sequestrum was followed by widespread erysipelas. Calcium was deposited in the stomach, and amyloidosis involved the spleen. His fifth case¹ concerned a man 73 years old, who was operated on for carcinoma of the lip and the cervical lymph nodes. The lungs, the kidneys, the other organs, the clavicles and a rib were sites of metastases. The lungs contained calcium deposits. Also present in Virchow's third, fourth and fifth cases was active degenerative nephritis in the late second or beginning third stage.

The first case reported by Virchow in a second paper⁴ was that of a woman 50 years old, who had a mammary cystosarcoma resected and recurring during two years. The thoracic wall and the adjacent lung were involved by neoplastic tissue. Metastases were seen in the following sites: lungs, mediastinum, liver, ribs, vertebrae, pelvic bones, dura, skull, cavernous sinus. Calcium deposits marked the lungs, the stomach, the kidneys, the rectum, the dura and the internal carotid artery.

Küttner's⁵ patient was a 19 year old woman who had abdominal pain and diarrhea for two months. Her spleen and liver were enlarged. She had fever, increasingly rigid peripheral arteries and terminal meningitis. Autopsy revealed: extensive rarefying osteitis or "scrofulous caries" of the thoracolumbar vertebrae; caseous tuberculosis of the meninges, the spleen, the liver and the kidneys; calcification of the systemic arteries; amyloidosis of the spleen and the liver.

The 48 year old man described by Litten⁶ suffered from loss of weight, anorexia, and pain and swelling of the extremities for a few months. He exhibited anasarca, a right submaxillary tumor, multiple

4. Virchow, R.: *Virchows Arch. f. path. Anat.* **9**:618, 1856.

5. Küttner: *Virchows Arch. f. path. Anat.* **55**:521, 1872.

6. Litten, M.: *Virchows Arch. f. path. Anat.* **83**:508, 1881.

pigmented nevi on the face, the neck and the trunk, dyspnea, oliguria and a low blood pressure. Metastatic alveolar round cell sarcoma, probably arising in a cancerous cutaneous melanoma, involved all viscera, especially the stomach, the intestines, the peritoneum, the heart, the kidneys, the vertebrae and the skeletal muscles. The kidneys and the cutaneous arteries contained calcium deposits.

Roth⁷ described a man 29 years old in whom osteomyelitis of the phalanges developed after traumatic amputation of the fingers. Incision and drainage were followed by nausea, vomiting, fever, delirium, drowsiness and albuminuria. The heart and the stomach were calcified, and the left renal vein and branches were thrombosed.

In a woman 26 years old, Haskoveč⁸ found cancer of the vagina, osteoporosis of bones caused by "capillary emboli" of cancer cells and calcification of the lungs and the kidneys.

A 15 year old youth described by Czech⁸ had a partly calcified sarcoma of the prostate gland, a paravesical abscess eroding a pubic bone, calcific deposits in the lungs and parenchymatous degeneration of the kidneys.

Plaue⁸ examined a woman 49 years old who had neoplasms of unspecified nature in the skull, the twelfth thoracic vertebra and a rib, calcification of the left lung and chronic interstitial nephritis.

Heller⁸ saw a patient with cancer of a vertebra, calcium deposits in the lungs and diseased kidneys. The renal lesion was not defined.

Kockel's⁹ first case was that of a woman 35 years old who had had a vaginal hysterectomy for cervical cancer three years before. For one year she was afflicted with gradual paralysis of the lower extremities. Marasmus, anemia and sacral decubitus were terminal. Autopsy showed: metastatic squamous cell carcinoma of the thoracolumbar vertebrae, the pelvic bones, the liver, the lungs and the pleura; calcification of the lungs; calculi of the left kidney; bilateral hydronephrosis; organizing and recent pneumonia; cancer cell embolism and thrombosis of pulmonary arteries. His second case⁹ concerned a woman 54 years old, who had undergone hysterectomy for cervical carcinoma. Autopsy disclosed metastatic squamous cell carcinoma in the left iliac bone, in the lumbar vertebrae and in the lungs, calcium deposits in the lungs and emboli of cancer cells in pulmonary arteries.

The first case of Davidsohn¹⁰ was that of a man 27 years old, who had a sarcoma of the first sacral vertebra with invasion of the adjacent vertebrae and of the pelvic bones and metastases in the lungs. Calcium deposits marked the heart, the lungs and the kidneys. His second

7. Roth, M.: *Cor.-Bl. f. schweiz. Aerzte* **14**:226, 1884.

8. Cited by Askanazy.²

9. Kockel, R.: *Deutsches Arch. f. klin. Med.* **64**:332, 1899.

10. Davidsohn, C.: *Virchows Arch. f. path. Anat.* **160**:538, 1900.

patient ¹⁰ was a 32 year old man with a gastric carcinoma metastasizing to the pelvic bones, a humerus and a femur, calcification of the left lung, the stomach and the kidneys, and fat embolism of the lungs.

Askanazy's first patient ² was a man 37 years old, with a large round cell sarcoma (obviously a cancerous melanoma) of the right thigh, which metastasized to the following sites: right inguinal lymph nodes, skin, heart, liver, left adrenal gland and kidney; pancreas, sternum, ribs, dnra, frontal bone, second lumbar vertebra, left iliae bone. Also observed were calcification of the stomach, pulmonary emphysema, purulent bronchitis, hemorrhagic nephritis and thrombosis of renal veins. His second case ² concerned a woman 54 years old, who had a goiter for five years. For one year, after trauma of her back, she suffered sacral pain. For three weeks she had renal colic and passed two calcium sulfate stones. Terminally her urine contained leukocytes and coliform organisms, and she was afflicted with vomiting, fever, and renal pain in the left side. In the ribs, the vertebrae and the sternum was found "progressive bone atrophy," characterized by osteoclasts, osteoporosis and fibrosis of marrow. Also present were carcinoma of a substernal thyroid gland with metastases in the lungs, calcium deposits in the lungs and the kidneys, calcified thrombi in the pulmonary arteries, renal calculi, purulent pyelitis and organizing pneumonia.

Bender's first patient ¹¹ was a youth 14 years old, who had arthritis of the shoulders, the elbows, the hands, the ankles and the feet. A nodule removed from the patella for biopsy was diagnosed as lymphosarcoma. Also observed were: a primary round cell sarcoma of the periosteum of the left femur with metastases in ribs, vertebrae, femurs, tibias, fibulas, humeri, lymph nodes, kidneys and right testis; calcification of the lungs, the stomach, the liver and the kidneys; lobular pneumonia. His second patient ¹¹ was a man 51 years old, who suffered from sacral pain, melena, constipation and paresthesias of the legs for three months. This patient was emaciated, dipsomania and unable to walk. He showed deformities of the thoracolumbar vertebrae, paralysis of the lower extremities, sacral decubital ulcer and albuminuria. Plasma cell myeloma involved skull, ribs, vertebrae, left clavicle, pelvic bones, sacrum, scapula, right humerus and right femur. Calcification of the lungs, compression of the sacral plexus, embolism of a branch of the right pulmonary artery and bilateral cystoureteropyelonephritis were also present.

Stokvis ¹² described a 39 year old man with Bence Jones protein in the urine and the feces. A diffuse bone disease, called "osteosarcomatosis," but probably multiple myeloma, and calcification of the kidneys were found.

11. Bender, O.: *Deutsche Ztschr. f. Chir.* 63:370, 1902.

12. Stokvis, B. I., cited by Parkes-Weber, F. A.: *Med.-Chir. Tr.* 86:395, 1903.

For seven months a man 50 years old, described by Scheele and Herxheimer,¹³ suffered from pain in the chest, the back, the joints and the muscles. Two months before, he had bronchitis and pneumonia. He showed fever, recurrent pneumonia, spontaneous fracture of the left femur, albuminuria and swelling of the right cervical and left inguinal regions. Multiple myeloma of the left femur, the ribs, the sternum, the clavicles and the vertebrae, calcification of the kidneys, bronchopneumonia and nephritis were found.

A woman 19 years old, described by Lazarus and Davidsohn,¹⁴ suffered from fever, night sweats, pain in the feet, the right leg and the shoulders, and swelling of the right knee. She exhibited a systolic murmur, paralysis of the left abducens nerve, dyspnea, prostration, splenomegaly, albuminuria and pulmonary rales. A meningeal sarcoma invaded the skull bones, and the heart, the lungs, the stomach, the kidneys and the aorta were calcified.

Huebschmann¹⁵ reported the case of a woman 26 years old, who showed the following conditions: a carcinoma of the vagina with erosion of the pelvic bones, invasion of the bladder and the rectum, and compression of the ureters; calcification of the lungs and the kidneys; calcific thrombosis of an artery and a calcified infarct in the lower lobe of the right lung, and bronchopneumonia.

For five years a woman 36 years old, described by Tschistowitsch and Kolessnikoff,¹⁶ suffered from pains in the neck, the trunk and the extremities. For six months she had dyspnea, vomiting and constipation. She showed elevated vital signs, emaciation, albuminuria with Bence Jones protein, and terminal pneumonia. Autopsy disclosed: myeloblastic myeloma in the ribs, the sternum, the clavicles, the ilium and the vertebrae; calcification of the heart, the systemic arteries, the lungs, the stomach and the kidneys; organizing pneumonia.

For three months, following trauma, a boy 12 years old, described by Jadassohn,¹⁷ had pain in the trunk, polydipsia, loss of weight and vomiting. The following abnormalities were observed clinically: cutaneous nodules over the elbows and knees; subcutaneous stripes; vesicles, pustules and abscesses in the skin; high grade destruction of the pelvic bones, revealed on roentgen examination; a blood culture showing staphylococci; a terminal high fever. Autopsy disclosed: osteomyelitis of the right ilium and of the ribs; rarefaction of the left ilium and of the tibial epiphyses; calcium deposits in the heart, the lungs,

13. Scheele and Herxheimer: *Ztschr. f. klin. Med.* **54**:57, 1904.

14. Lazarus, P., and Davidsohn, C.: *Ztschr. f. klin. Med.* **60**:314, 1906.

15. Huebschmann, P.: *Centralbl. f. allg. Path. u. path. Anat.* **19**:737, 1908.

16. Tschistowitsch, T., and Kolessnikoff, H.: *Virchows Arch. f. path. Anat.* **197**:112, 1909.

17. Jadassohn, J.: *Arch. f. Dermat. u. Syph.* **100**:317, 1910.

the splenic arteries, the kidneys and the skin; staphylococcic aortic valvulitis; multiple pulmonary, myocardial and renal abscesses (left kidney).

Pari¹⁸ reported the case of a woman 25 years old, with the following conditions: carcinoma of the uterine cervix; metastases in lumbar vertebrae, the ovaries, the liver and lumbar lymph nodes; extension to the ureters with obstructive hydronephrosis; extension to the bladder with perforation; calcification of the lungs; organizing pneumonia.

A man 25 years old, described by Versé,¹⁹ had chronic myelogenous leukemia for thirty months. Typical lesions involved the marrow, the spleen and the liver. Also present were: extensive destruction of bone; calcification of the heart, the systemic arteries, the lungs and the kidneys; mural thrombosis of the left auricle.

Schober²⁰ examined a patient with a destructive inflammatory process, probably osteomyelitis, of the calvarium and calcification of the heart and the lungs.

Wells²¹ reported the case of a 30 year old man who had: myelogenous leukemia with typical lesions in the sternum, the clavicles, other bones, the liver, the kidneys and other organs; calcification of the heart, the coronary arteries, the lungs and the kidneys; a patent foramen ovale.

For two months the 52 year old woman described by Froboese²² had a tender mass in the right lumbar region. A severely pyonephrotic right kidney was removed at operation. She began to have swollen, painful feet, pallor, enlargement of the heart, bronchitis and albuminuria. A multiple myeloma (*Erythroblastom*) was found involving the sternum, the ribs, the vertebrae, the femurs and other bones, with halisteresis, osteoporosis and osteoclastosis, and calcification of the lungs and the kidneys.

Schulze's²³ patient was a boy 11 years old, who suffered pain and limitation of motion of the vertebrae and of the extremities for one year. He displayed emaciation, pallor, weakness, thoracic scoliosis, a rigid spine, rachitic teeth, cutaneous and tendinous calcium deposits, hard peripheral arteries, calcified arteries in the extremities by roentgenogram, slight albuminuria and terminal cardiac failure. Osteopetrosis (Albers-Schönberg disease) of the skeletal bones was found, and calcium deposits in the heart, the systemic arteries, the lungs, the stomach, the kidneys and the periarticular structures of the hip, the knee and the vertebral joints.

18. Pari, G. A.: Virchows Arch. f. path. Anat. **200**:199, 1910.

19. Versé, M.: Verhandl. d. deutsch. path. Gesellsch. **14**:281, 1910; Centralbl. f. allg. Path. u. path. Anat. **21**:459, 1910.

20. Schober, cited by Stumpf: Centralbl. f. allg. Path. u. path. Anat. **25**:801, 1914.

21. Wells, H. G.: Arch. Int. Med. **15**:574, 1915.

22. Froboese, C.: Virchows Arch. f. path. Anat. **222**:291, 1916.

23. Schulze, F.: Mitt. a. d. Grenzgeb. d. Med. u. Chir. **36**:243, 1923.

For two years the 46 year old woman observed by Barr and Bulger²⁴ had night sweats and lassitude. Examination showed: fever; emaciation; pallor; rarefaction of the ribs, the lumbar vertebrae and the pelvic bones by roentgenogram; a hemoglobin content of 48 per cent; erythrocytes 3,250,000 per cubic millimeter; serum calcium 16 mg. and serum phosphorus 3.7 mg. per hundred cubic centimeters; blood nonprotein nitrogen 70 mg. per hundred cubic centimeters; albuminuria with hyaline and granular casts; excretion of 51 per cent of injected phenolsulfonphthalein in two hours; serum calcium 17.8 mg. per hundred cubic centimeters with a high calcium intake; terminal pneumonia. Autopsy revealed: plasma cell myeloma in the ribs, the clavicles and the vertebrae; calcification of the lungs, the stomach and the kidneys; bronchopneumonia; chronic nephritis; calculi of the gallbladder and the cystic duct.

The 19 year old woman described by deSanto²⁵ had suffered weakness, anorexia and loss of weight for one year. Ten months before she was admitted to the hospital she had an acute rash, pruritus, splenomegaly and a blood picture typical of myelogenous leukemia. Examination showed: pallor; emaciation; albuminuria; a basal metabolic rate of +75 per cent; 21,200 to 1,200,000 blood leukocytes per cubic millimeter, with varying percentages of immature neutrophilic granulocytes; terminal pulmonary rales, toxemia and coma. Typical lesions of myelogenous leukemia involved the bone marrow, the spleen, the liver, the kidneys and lymph nodes. Calcification was present in the heart, the systemic arteries, the lungs and the kidneys. The lungs contained multiple arterial emboli and infarcts.

Egoville²⁶ reported the case of a woman 30 years old. Her fourth pregnancy had begun nine months before. For three months she noticed progressive weakness and severe pain in the shoulders, the back and the lumbar region. One month before examination she was delivered of a macerated fetus, and roentgenograms showed punched-out areas in the calvarium, the vertebrae, the femurs and the humeri. Biopsy of a rib showed metastatic adenocarcinoma. The serum calcium was 18.2 mg. and the serum phosphorus 9 mg. per hundred cubic centimeters, and the total serum protein was 5.5 Gm. per hundred cubic centimeters. Gastric analysis revealed achlorhydria. Adenocarcinoma of the right breast with metastases to vertebrae, ribs, liver and lymph nodes and calcification of the heart, the lungs and the kidneys were demonstrated at autopsy.

For two months the 13 year old girl described by Grayzel and Lederer²⁷ had pain in the thighs, radiating to the ankles and the feet,

24. Barr, D. P., and Bulger, H. A.: *Am. J. M. Sc.* **179**:449, 1930.

25. DeSanto, D. A.: *Am. J. Path.* **9**:105, 1933.

26. Egoville, J. W.: *Arch. Path.* **26**:1047, 1938.

(Footnotes continued on next page)

pain in the flanks, vomiting and loss of weight. Examination disclosed: a palpable right kidney; a tender left twelfth rib; rarefaction and mottling of the shafts of the fibulas and of the thoracolumbar vertebrae and punched-out areas in the humeri; slight albuminuria; a phenolsulfonplithalein test with excretion of 27 per cent; a blood hemoglobin content of 60 per cent; 3,400,000 erythrocytes per cubic millimeter; 6,300 leukocytes per cubic millimeter, with 85 per cent neutrophils and 15 per cent lymphocytes; plasma chlorides 424 mg., blood nonprotein nitrogen 101 mg., serum albumin 3.5 Gm., serum globulin 2.8 Gm., serum cholesterol 197 mg., serum calcium 20.6 mg., serum phosphorus 4.7 mg., serum phosphatase 4.5 units²⁷ and blood sugar 134 mg. per hundred cubic centimeters; carbon dioxide-combining power 53.5 volumes per cent. Also found were aplastic myelosis, involving ribs, vertebrae, spleen, liver, lymph nodes, kidneys and thymus, and calcification of the heart and the lungs.

Wells and Holley²⁸ reported the case of a man 59 years old, who noticed an enlarging head for nine years, bowed legs for five years, weakness and loss of weight for four years, pain and stiffness in the left hip and knee for three years and alopecia, diplopia and decreased visual acuity for eight months. Examination showed: a large sternum; flared ribs; a barrel chest; a systolic murmur; pulmonary rales; spinal kyphosis; *enlarged, thickened, moth-eaten calvarium, mandible, and bones of extremities*, variation in the density of the vertebrae and enlarged pelvic bones by roentgenogram; serum calcium 10 to 11.8 mg., serum phosphorus 3.7 to 4.5 mg., serum potassium 16.7 to 17.9 mg. per hundred cubic centimeters; a blood p_{H} of 7.3 to 7.45. He received 5,000,000 units of viosterol in two weeks and died of terminal bronchopneumonia. Osteitis deformans was found in the skull, the sternum, the ribs, the vertebrae, the iliac bones, the left femur and the left tibia, and calcium deposits in the heart, the lungs, the stomach, the kidneys and the skin.

Summary.—Of 35 patients having some type of bone disease and metastatic calcification, 17 were of the male and 18 of the female sex. The ages of 34 were between 11 and 73 years, the disease occurring before the age of 40 in 23 and after the age of 60 in only 1. The lesions of the bones represented metastatic carcinoma in 7 cases, multiple myeloma in 6, osteomyelitis in 5, myelogenous leukemia and unspecified cancer in 4 each, metastatic sarcoma in 3, primary sarcoma and metastatic melanoma in 2 each, and tuberculosis, "progressive bone atrophy," osteopetrosis (Albers-Schönberg disease) and osteitis deformans in 1 each. In 1 case of osteomyelitis⁸ metastatic bone sarcoma was probably

27. Grayzel, D. M., and Lederer, M.: Arch. Int. Med. 64:136, 1939.

27a. In all instances in which the type of unit is not stated this is due to the fact that the type was not specified in the article reviewed.

28. Wells, H. G., and Holley, S. W.: Arch. Path. 34:435, 1942.

present. In Bender's¹¹ first patient with primary sarcoma of bone, probably Ewing's sarcoma, metastatic bone sarcoma was widespread. As a contributing cause of metastatic calcification, some type of nephropathy was found in 15 cases, including 3 reported by Virchow,¹ those of Küttner,⁵ Czech,⁸ Plaue⁸ and Heller,⁸ Kockel's⁹ first case, those of Askanazy,² Bender's¹¹ second case and those of Scheele and Herxheimer,¹³ Jadassohn,¹⁷ Pari¹⁸ and Barr and Bulger.²⁴ In the other 22 cases nephropathy other than renal calcification was not a factor so far as could be determined from the available protocols. Associated dystrophic calcification of the lungs was definitely a factor to be considered in both of Kockel's⁹ cases, Askanazy's² second case, Huebschmann's¹⁵ case and Pari's¹⁸ case. The parathyroid glands were examined in only 4 cases²⁹ and were found to be normal. Hypervitaminosis D probably played some role in the calcification of the tissues of the patient observed by Wells and Holley.²⁸

Calcium deposits were found in the lungs in 30 cases, most often in alveolar walls, veins, arteries, capillaries, bronchi and bronchioles. Elastic fibrils in alveolar walls, veins and arteries were often the sites of calcification. The veins were usually more heavily calcified than the arteries. Less common locations for calcific deposits were the stroma, the bronchial cartilages and the lumens of alveoli and blood vessels.

In 21 cases calcium deposits were found in the kidneys, most often in the lumens, cells and basement membranes of convoluted and collecting tubules and, to a much less extent, of Henle's loops. Also calcified with significant frequency were the renal stroma and the basement membranes of glomeruli. Other less common locations for calcium deposits included arteries, arterioles and capillaries. Thrombosis of the renal veins was observed in 2 instances,³⁰ but calcium was not described in the renal veins in any case.

In 13 cases the heart contained calcium deposits, preponderantly in the endocardium of the left auricle, involving especially elastic fibrils. In a few cases the endocardium and the myocardium of the left ventricle showed calcified elastic fibrils and muscle fibers. The mitral and aortic valves were also involved by calcification in a few instances. The right chambers of the heart were little if any involved.

In 12 cases the systemic arteries were calcified, including the abdominal aorta, the coronary, mesenteric, femoral, hepatic, renal, splenic, carotid, pancreatic and cutaneous arteries, and the arteries of the extremities. The calcium salts were found as fine granules or plaques in an otherwise normal intima, often in relation to the internal elastic lamella, and in the elastic fibrils, or sometimes in the muscle fibers, of the media.

29. Barr and Bulger.²⁴ Egoville.²⁶ Grayzel and Lederer.²⁷ Wells and Holley.²⁸

30. Askanazy.² Roth.⁷

In 10 cases the stomach contained calcium deposits, most notably in the interglandular stroma of the fundic mucosa and to a lesser degree in gland cells and lumens, and in capillaries.

Miscellaneous sites of calcification included the skin,³¹ the rectum,⁴ the dura,⁴ periarticular structures³² and the liver.¹¹ In Bender's first case¹¹ the calcification of the liver was most likely dystrophic in nature.

Chemical analysis showed calcium carbonate in the lungs of 5 patients,³² in the hearts of 3,³³ in the kidneys of 2³⁴ and in the gastric mucosa,⁷ the skin¹⁷ and the renal calculi¹¹ of 1 each. Calcium phosphate was found in the lungs,³² the gastric mucosa,¹⁶ the heart,²¹ the kidney²¹ and in renal calculi.¹ Calcium sulfate was found in the renal calculi of 1 patient.²

METASTATIC CALCIFICATION AND CHRONIC RENAL DISEASE

The second case of metastatic calcification reported by Virchow¹ was that of a woman 43 years old who had had pleuritis two months before and then suffered from bleeding hemorrhoids. The clinical findings were: fever; anasarca; bronchitis; abdominal pain; polyuria; casts, erythrocytes and leukocytes in the urine; terminal erysipelas. vomiting and dyspnea. Autopsy revealed active degenerative nephritis in the late second or beginning third stage of the disease; calcification of the left lung; pulmonary edema; an infarct of the left kidney; left serofibrinous pleuritis.

The kidneys of a man 44 years old, described by Stade,⁸ showed interstitial nephritis, and both lungs and kidneys contained calcium deposits.

Bryant and White³⁶ recorded the case of a 6 month old boy who had suffered from loss of weight, weakness and constipation for three months. He showed a scruffy, alopecic scalp, a few basal rhonchi in the lungs, vomiting, diarrhea, and terminal gangrene of the right foot. Autopsy disclosed: obstructive cystoureteropyelonephritis with hydro-nephrosis; extreme phimosis; calcification of the heart and the systemic arteries; nodular tuberculosis of the lungs, the bronchial lymph nodes and the spleen; thrombosis of the right anterior tibial artery.

In a woman 36 years old, Schmidt³⁷ found: chronic interstitial nephritis; renal endarteritis; calcification of the heart, the systemic arteries, the splenic veins, the lungs and the stomach; mural thrombosis of the cardiac ventricles; embolic hemorrhagic infarcts of the upper lobes

31. Jadassohn.¹⁷ Wells and Holley.²⁸

32. Koekel.⁹ Bender.¹¹ Jadassohn.¹⁷ Pari.¹⁸ Wells.²¹

33. Roth.⁷ Jadassohn.¹⁷ Wells.²¹

34. Roth.⁷ Wells.²¹

35. Bender.¹¹ Tschistowitsch and Kolessnikoff.¹⁶ Wells.²¹

36. Bryant, J. H., and White, W. H.: *Guy's Hosp. Rep.* 55:17, 1901.

37. Schmidt, M. B.: *Deutsche med. Wchnschr.* 39:59, 1913.

and atelectasis of the lower lobes of the lungs; chronic passive hyperemia of the liver; cholesterolosis of the gallbladder; vaginal ulcers; hyperplastic nodules in the thyroid gland; a calcified right bronchial lymph node.

A man 20 years old, described by Hubbard and Wentworth,³⁸ had masses around the larger joints of the extremities for eleven months. Examination showed: large calcium deposits around these joints and calcified arteries by roentgenogram; calcified media in a biopsy specimen of a peripheral artery; a serum calcium content of 13.4 mg. per hundred cubic centimeters, falling to 11.9 mg. during three days of a low calcium diet and rising to 12.7 mg. during three days of a high calcium diet; other blood findings typical of progressive chronic nephritis. Autopsy disclosed: severe chronic interstitial nephritis; right hydronephrosis; calcification of the heart, the small peripheral arteries, the periarticular structures, the jejunum and the gastroepiploic omentum; osteitis fibrosa, especially in the skull, the ribs and the vertebrae; hyperplasia of two enlarged parathyroid glands, one inclosing a small adenoma.

Butler's³⁹ first patient was a Negro woman 38 years old, who suffered from dyspnea and postprandial emesis. Examination showed: emaciation; an enlarged heart with diastolic gallop and apical systolic murmur; rales in the left lung; hepatomegaly; edema of the ankles; a blood pressure of 220 systolic and 160 diastolic; blood urea 5.6 Gm. per liter; albuminuria with many leukocytes; terminal uremia. At autopsy chronic nephritis, calcification of the left lung, edema of the right lung, organizing pneumonia, cardiac hypertrophy and an infarct of one kidney were found. His second patient was a woman 31 years old, who had suffered from headaches, palpitation, bilious attacks and edema of the legs and feet for eight years. Examination showed: poor nurture; an enlarged heart; pulmonary rales; albuminuria; a phenol-sulfonphthalein test with excretion of less than 10 per cent; blood urea 1.43 to 3.63 Gm., per liter. Also present were chronic nephritis, calcium deposits in the lungs, edema of the lungs and right fibrinous pleuritis.

Müller⁴⁰ recorded the case of a man 20 years old, who had had his right femur amputated for tuberculous osteomyelitis seven years before. For one year he suffered from purulent streptococcic pyelocystitis, and died in uremia. Autopsy disclosed: pyonephrosis of the left kidney; chronic left ureteritis; chronic cystitis; suppurative pericystitis; amyloidosis of the right kidney and of the spleen, the liver and the heart; calcium deposits in the heart, the systemic arteries and the right kidney; tuberculosis of the left lung, the bronchial lymph nodes, the spleen and the liver.

38. Hubbard, R. S., and Wentworth, J. A.: *Proc. Soc. Exper. Biol. & Med.* **18**:307, 1921.

39. Butler, M.: *Proc. New York Path. Soc.* **24**:79, 1924.

40. Müller, H.: *Klin. Wchnschr.* **5**:1703, 1926.

The 27 month old girl described by Lightwood⁴¹ suffered from stunting of growth and intermittent vomiting. She had facial paralysis twenty-two months before; a cardiac murmur one year before, and failure to gain weight, dysphagia, flatulence and inability to walk for one year. For ten days before admission she received 2 minims (0.12 cc.) of a preparation of vitamin D daily. She exhibited dwarfism, retarded mentality, pallor, poor nurture, genu valgum and carious teeth. Examination showed: a systolic blood pressure of 180; hard tortuous arteries in the extremities, visible by roentgen ray; narrowed retinal arterioles; paresis of the right side of the face and of the left external rectus muscle; albuminuria; an average urea concentration of 1.5 per cent in two hours; blood urea 169 mg., blood cholesterol 196 mg., serum calcium 11 mg. and serum phosphorus 6.7 mg. per hundred cubic centimeters; terminal fever. Autopsy revealed: chronic nephritis; calcium deposits in the heart, the systemic arteries, the lungs, the kidneys, the trachea, the parietal pleura, the dura and the tentorium; rickets in the long bones; pulmonary edema; bronchopneumonia.

Albright, Baird, Cope and Bloomberg⁴² described a man 23 years old, who suffered from nausea, weakness, vomiting, polyuria and nocturia. He had a blood pressure of 140 systolic and 100 diastolic, 2,500,000 erythrocytes per cubic millimeter, 185 mg. of urea nitrogen per hundred cubic centimeters of blood and albuminuria. Chronic nephritis, calcification of the lungs and the kidneys, osteoporosis and osteoclastosis of the vertebrae and lobular pneumonia were observed.

Platt and Owen⁴³ reported the case of an 18 year old youth who had been afflicted with weakness, debility, polydipsia, polyuria, nocturia, drowsiness and mental dullness since early childhood. For three years he had genu valgum and anorexia. He displayed dwarfism, emaciation, pallor and pigmented skin raised in hard plaques in the axillary and inguinal regions. Examination showed: an enlarged heart; a blood pressure of 155 systolic and 100 diastolic; albuminuria; a hemoglobin content of 30 per cent; 1,560,000 erythrocytes and 17,900 leukocytes per cubic millimeter; an increased sedimentation rate of the red blood cells; blood nonprotein nitrogen 333 mg., blood urea nitrogen 205 mg., plasma cholesterol 179 mg., serum calcium 6.7 mg. and serum phosphorus 15.1 mg. per hundred cubic centimeters; bone erosion in the spine and sacrum, rickets in the bones of the extremities, and calcification of the arteries and of the pigmented cutaneous areas by roentgenogram. Terminally there were drowsiness, dyspnea, convulsions and uremia, with the blood nonprotein nitrogen 400 mg. per hundred cubic centi-

41. Lightwood, R.: *Arch. Dis. Childhood* 7:193, 1932.

42. Albright, F.; Baird, P. C.; Cope, O., and Bloomberg, E.: *Am. J. M. Sc.* 187:49, 1934.

43. Platt, R., and Owen, T. K.: *Lancet* 2:135, 1934.

meters. Chronic nephritis, calcification of the lungs, the splenic blood vessels, and the skin, cardiac hypertrophy and pulmonary edema were also found.

A boy 14 years old, described by Smyth and Goldman,⁴⁴ sustained a streptococcic infection of a finger, followed by lymphangitis, lymphadenitis, bacteremia, nephritis and anemia. Eighteen months later he had a waddling gait and stiffness and weakness of the lower extremities. Two years after the onset of the infection he exhibited a poor posture, emaciation, pallor, increased anteroposterior diameter of the chest and flaring of the costal margins. Examination revealed: an enlarged heart; a systolic murmur; hard radial arteries; a blood pressure of 110 systolic and 56 diastolic; a palpable liver; albuminuria; low urinary output; 3 to 7 per cent excretion of injected phenolsulfonphthalein in two hours; urea clearance of 3.4 to 18.1 per cent; a hemoglobin content of 30 to 55 per cent; 1,500,000 to 2,860,000 erythrocytes per cubic millimeter; 4,760 to 6,720 leukocytes per cubic millimeter, with neutrophils 50 to 87 per cent, lymphocytes 10 to 33 per cent and monocytes 2 to 8 per cent; serum albumin 4.5 Gm., serum globulin 1.5 Gm., blood nonprotein nitrogen 80 to 240 mg., plasma chlorides 455 mg., serum calcium 10.5 to 11.9 mg., serum phosphorus 10.5 to 16.0 mg. and serum phosphatase 7.5 to 10.7 units per hundred cubic centimeters, and carbon dioxide-combining power 49.6 volumes per cent; anorexia; dyspnea; asthenia; cough; a mass on the right coracoacromial joint; tumors on the sternoclavicular and phalangeal joints; dry, brittle, transversely ridged nails; a negative calcium balance with a low calcium intake, a positive calcium balance on a high calcium intake, and a great retention of phosphorus with a high phosphorus intake. Terminally there were pruritus, rigid arteries, epistaxis, nausea, vomiting, prostration and convulsive seizure. Autopsy disclosed: chronic hydronephrotic pyelonephritis; calcification of the heart, the systemic arteries, the lungs, the stomach, the kidneys, the periarticular structures and the dura; rickets of the long bones; otitis media on the right, due to infection with coliform organisms and pneumococci; terminal streptococcic and staphylococcic bacteremia.

The patient observed by Shelling and Remsen⁴⁵ was a youth 17 years old, who had gradual onset of deformities of all four extremities and of the chest and inability to walk for three years. Three months before admission he fractured his right femur and was confined to bed. In addition to the changes mentioned, examination showed: pallor; hard peripheral arteries; narrowing of the arterioles in the ocular fundi; a hemoglobin content of 8.6 Gm. per hundred cubic centimeters of blood; 3,200,000 erythrocytes per cubic millimeter; 10,000 leukocytes

44. Smyth, F. S., and Goldman, L.: *Am. J. Dis. Child.* 48:596, 1934.

45. Shelling, D. H., and Remsen, D.: *Bull. Johns Hopkins Hosp.* 57:158, 1935.

per cubic millimeter, with 53 per cent segmented neutrophils and 42 per cent lymphocytes; serum calcium 9.3 to 9.9 mg., phosphorus 7.7 to 10.5 mg., phosphatase 22.1 Bodansky units, blood nonprotein nitrogen 153 to 364 mg., blood cholesterol 208 mg. and total serum protein 4.7 to 5.9 Gm. per hundred cubic centimeters; an albumin-globulin ratio of 1.7; blood chlorides 76 to 113 milliequivalents; carbon dioxide-combining power 27 to 48 volumes per cent; normal sugar tolerance and basal metabolic rate; urine loaded with erythrocytes and leukocytes; a phenolsulfonphthalein test with excretion of less than 5 per cent in two hours; calcification of the arteries in the fingers and subcutaneous tissue, halisteresis and deformities of skeletal bones, and osteoporosis of long bones by roentgenogram; a positive test of the blood for parathyroid hormone. Terminally anorexia, drowsiness, vomiting, dehydration, acidosis, streptococcal bacteremia and stupor were observed. Post mortem chronic suppurative hydronephrotic pyelonephritis, calcification of systemic arteries, abscesses of the lungs and the liver and focal necrosis of the anterior lobe of the pituitary gland were found.

The 23 year old woman described by Magnus and Scott⁴⁶ had increasing weakness, exertional dyspnea, drowsiness, loss of weight, manual tremors, clubbed fingers, cold feet, scoliosis and brown pigmentation of the skin for ten months. In addition, examination showed: emaciation; an enlarged thyroid gland; a blood pressure of 96 systolic and 62 diastolic; hard peripheral arteries; thick subcutaneous tissue in the legs; a hemoglobin content of 43 per cent; serum calcium 11 mg. and serum sodium 319 mg. per hundred cubic centimeters; calcified arteries in the extremities and subcutaneous calcium deposits in the legs by roentgenogram. Terminally there were vomiting and coma. Chronic nephritis and calcification of the systemic arteries and of the subcutaneous tissue of the legs were observed.

Pollack and Siegal⁴⁷ reported the case of a woman 41 years old who had recurrent pain in the thighs for eighteen months, general pruritus, pigmentation of the skin, polyuria and nocturia for one year, and subcutaneous swellings, loss of weight and pain in the shoulders for six months. Examination showed: a blood pressure of 153 systolic and 76 diastolic; bilateral Babinski signs; a Chaddock sign on the left; subcutaneous nodules on the hands, the right elbow, the left infrascapular region, the left knee and the toes; a hemoglobin content of 53 per cent; erythrocytes 3,400,000 and leukocytes 4,300 per cubic millimeter; blood urea nitrogen 95 mg., serum calcium 12 to 13 mg., phosphorus 4 to 6.3 mg. and phosphatase 5 Bodansky units per hundred cubic centimeters; carbon dioxide-combining power of the blood 35 volumes per cent; albuminuria; no excretion of intramuscularly injected phenolsulfon-

46. Magnus, H. A., and Scott, R. B.: *J. Path. & Bact.* 42:665, 1936.

47. Pollack, H., and Siegal, S.: *J. Mt. Sinai Hosp.* 2:270, 1936.

phthalein in two hours; a basal metabolic rate of + 57 to 63 per cent; widespread calcium deposits in the shoulders, the hands, the buttocks, the pelvic blood vessels and the left foot by roentgenogram; heavy calcium deposits in a toe by biopsy; elevated serum calcium after 15 Gm. of calcium gluconate had been taken orally, indicating an increase of the blood parathyroid hormone. She received strong solution of iodine, U.S.P., and viosterol by mouth prior to a partial resection of enlarged parathyroid glands, which showed diffuse hyperplasia. Most of the thyroid gland was also resected, but it failed to reveal hyperplasia. The serum calcium fell to 10.2 mg. per hundred cubic centimeters, the blood urea nitrogen increased, oliguria developed, and high terminal fever was observed. Chronic nephritis, calcification of the lungs and the kidneys, healed tuberculosis of the lungs and the bronchial lymph nodes, two small pheochromocytomas of the adrenal medulla and cholelithiasis were demonstrated.

Price and Davie⁴⁸ recorded the case of a youth 14 years old who was deaf and who had suffered from polydipsia and polyuria for eleven years and an increasing deformity of the lower extremities with limitation of activity for six years. He exhibited stunting of stature, coarse, sparse hair, a depressed nose, large maxillas, a receded mandible, small scrotal testes and infantile secondary sex characteristics. The blood pressure was 120 systolic and 75 diastolic. There were flexion deformities of the hips, lumbar lordosis, waddling gait and genu valgum. The skeletal bones showed osteoporosis and enlarged metaphyses; the femurs, the tibiae and the fibulas, bowing, and the skull a thick, rarefied, honeycombed appearance, by roentgenogram. There was slight albuminuria, with 0.85 per cent urea concentration in three hours. Blood urea was 39.7 to 318 mg., serum calcium 12.5 to 13.6 mg., serum phosphorus 5 to 6.5 mg. and serum phosphatase 51.1 to 59.6 units per hundred cubic centimeters. Terminally twitchings, convulsions and coma were observed. Chronic nephritis, calcification of a kidney and rickets of a femur were also present.

Castleman and Mallory⁴⁹ described a man 45 years old who had scarlet fever thirty-five years before, a diagnosis of Bright's disease twenty years before, and cutaneous pruritus, nocturia and swollen fingers for thirty months. Examination showed: a blood pressure of 165 systolic and 90 diastolic; precordial systolic and diastolic murmurs; firm, tortuous peripheral arteries; cystic swelling of the right forefinger; a hemoglobin content of 65 per cent; erythrocytes 3,800,000 and leukocytes 12,000 per cubic millimeter, with segmented neutrophils 63 per cent; blood nonprotein nitrogen 130 mg., serum calcium 10.1 mg.,

48. Price, N. L., and Davie, T. B.: *Brit. J. Surg.* **24**:548, 1937.

49. Castleman, B., and Mallory, T. B.: *New England J. Med.* **214**:320, 1936; *Am. J. Path.* **13**:553, 1937.

phosphorus 7.9 mg., phosphatase 9.4 Bodansky units, serum protein 4.9 Gm. and blood uric acid 4.4 mg. per hundred cubic centimeters; blood chlorides 107 milliequivalents; carbon dioxide-combining power of blood 37.8 volumes per cent.; moderate albuminuria; a phenol-sulfonphthalein test with excretion of less than 15 per cent in one hour; masses of calcium around the finger, acromioclavicular and elbow joints, calcified blood vessels, and a skull marked by decalcified areas by roentgenogram; small kidneys by pyelogram. There were diarrhea and epistaxis, and terminally, severe pain in the chest, bundle branch block and blood pressure of 60 systolic and 50 diastolic. At autopsy chronic glomerulonephritis, calcification of systemic arteries and peri-articular structures, coronary thrombosis and occlusion, and rheumatic aortic and mitral valvulitis with mitral stenosis were demonstrated.

Pons and Pappenheimer⁵⁰ reported the case of a 33 year old man who had gain of weight, polyphagia, polydipsia and polyuria for eleven years, albuminuria for nine years, morning nausea and occasional vomiting for twenty-eight months, and high blood pressure, easy fatigue and palpitation for eight months. The hemoglobin content was 62 per cent; the erythrocyte count, 2,690,000; the leukocyte count, 3,600, with 65 per cent segmented neutrophils and 40.5 per cent lymphocytes; the blood showed nonprotein nitrogen 100 to 134 mg., uric acid 5.7 to 8.7 mg., urea nitrogen 38.7 to 62.8 mg. and dextrose 142 mg. per hundred cubic centimeters in the two months before admission, when his basal metabolic rate was —22 per cent and he was afflicted with weakness, vertigo, vomiting and diarrhea. He showed pallor and had an enlarged heart; his blood pressure was 132 systolic and 92 diastolic; there was adiposity of the face, neck and trunk, and purple striae on the abdomen and thighs. Osteoporosis of the calvarium was revealed by roentgenogram. Terminally he suffered from dysphagia, dry cough, insomnia, congested lungs, tremors of the extremities and general aching. Autopsy disclosed: chronic nephritis; calcification of the heart, the systemic arteries, the lungs and the stomach; osteitis fibrosa cystica of the skull and the vertebrae; cardiac cirrhosis of the liver; hemosiderosis of the spleen.

These authors also outlined the case of a man 23 years old, who had renal symptoms for several years before autopsy disclosed subacute glomerulonephritis, metastatic calcification of the lungs and the kidneys, osteitis fibrosa in the bones, and parathyroid glands weighing 2.67 Gm. The renal calcium of this patient was 1,300 mg. per hundred grams of wet tissue, compared with a normal of 11 mg. per hundred grams.

The case of Brown and Ginsberg⁵¹ was that of a woman 55 years old who had perennial albuminuria after scarlet fever forty-six years

50. Pons, J. A., and Pappenheimer, A. W.: Puerto Rico J. Trop. Med. 13:115, 1938.

51. Brown, C. L., and Ginsberg, I. W.: Arch. Path. 30:108, 1940.

before. For fourteen months she experienced weakness, gain in weight, easy fatigue, exertional dyspnea and ulcers of the legs. She displayed pallor, a 2 cm. mass on the right side of the mandible, and a deformity of the chest. Examination showed: a blood pressure of 186 systolic and 94 diastolic; a systolic murmur and thrill; rigid peripheral arteries; slight albuminuria; less than 5 per cent excretion of intramuscularly injected phenolsulfonphthalein in two hours; 12 per cent urea clearance; a bleeding time of ninety seconds; a clotting time of five minutes; blood erythrocytes 2,460,000 per cubic millimeter, leukocytes 11,400 per cubic millimeter, 64 per cent of which were segmented neutrophils, platelets 195,000 per cubic millimeter, blood urea nitrogen 68 mg., blood creatinine 2.3 mg., blood sugar 71 mg., serum cholesterol 182 mg., serum calcium 10.8 to 12.5 mg., serum phosphorus 5.2 to 7.2 mg., serum phosphatase 11.6 Bodansky units and serum protein 4.7 Gm. per hundred cubic centimeters; carbon dioxide-combining power of plasma 30 volumes per cent; halisteresis of cranial and other skeletal bones, a cystic area in the mandible, calcification of arteries, trachea and bronchi, thoracic kyphos, pelvic deformity and periostitis of the femurs, by roentgenogram; a negative calcium balance in forty-eight hours; a basal metabolic rate of + 34 to 49 per cent. Terminally there was left ventricular failure. For at least five months before death the patient took a high calcium, low phosphorus diet and 3,600 units of vitamin D daily. Chronic glomerulonephritis, calcification of the heart and systemic arteries and pulmonary edema were demonstrated post mortem.

Herbert, Miller and Richardson⁵² described a 40 year old woman who had suffered from weakness, polyuria, nocturia and intermittent vomiting for four years after nephritis had been diagnosed in the last trimester of a pregnancy which terminated prematurely. For one year she had pains in the knee, ankle, wrist, elbow and spinal joints. For six months she had headaches, dyspnea, easy fatigue, edema of the ankles and pallor. Her skin was lemon yellow. Examination showed: a blood pressure of 180 systolic and 110 diastolic; a systolic thrill and murmur; rigid peripheral arteries; albuminuria; a hemoglobin content of 38 per cent; erythrocytes 1,940,000 and leukocytes 7,400 per cubic millimeter; blood urea 300 mg., serum calcium 11.7 mg., serum phosphorus 7.8 mg. and serum phosphatase 32.4 Jenner-Kay units per hundred cubic centimeters; calcification of arteries and calcific deposits around the acromioclavicular and phalangeal joints, by roentgenogram; no urinary excretion of a dye given intravenously. Terminally there was a downhill course with uremia. Autopsy disclosed: chronic glomerulonephritis; calcification of the heart, the systemic arteries and the periarticular structures; osteitis fibrosa cystica of the vertebrae, the clavicles, the fingers and the ribs.

52. Herbert, F. K.; Miller, H. G., and Richardson, G. O.: *J. Path. & Bact.* 53:161, 1941.

The first case described by Andersen and Schlesinger⁵³ concerned a boy 6 months old who had twitching of the extremities for four days before admission. Examination showed: muscle fibrillations in the extremities; a bulging left ear drum; a congested pharynx; a nasal discharge; a systolic murmur; a palpable liver; a hemoglobin content of 7.3 to 11 Gm. per hundred cubic centimeters; erythrocytes 1,900,000 to 3,570,000 per cubic millimeter; leukocytes 7,000 to 35,000 per cubic millimeter, with 52 per cent segmented neutrophils, 39 per cent lymphocytes, 6 per cent eosinophils and 2 per cent monocytes; serum calcium 7.4 to 11 mg., phosphorus 5.2 to 12.3 mg., serum phosphatase 6 to 7.3 Bodansky units, blood nonprotein nitrogen 45 to 122 mg., blood sugar 107 mg., blood urea nitrogen 56.7 to 71.7 mg., blood creatinine 2.3 mg., blood cholesterol 210 mg., serum albumin 3.8 to 4.4 Gm. and serum globulin 2.2 to 2.9 mg. per hundred cubic centimeters; blood chlorides 85.7 to 108.8 milliequivalents; carbon dioxide of blood 8.5 to 38.5 milliequivalents; slight albuminuria; *Bacillus lactis aerogenes* and *Streptococcus viridans* in the urine; puriform fluid with left myringotomy; low grade fever; episodes of vomiting; calcified blood vessels in the extremities and osteoporosis of the ends of the long bones by roentgenogram. Terminally he presented craniotabes, hard peripheral arteries, diffuse precordial systolic murmur and high fever. For one month before admission he received 500 units of vitamin D₃ as percomorph liver oil daily. For the next five months he was given 170,000 units of vitamin D₃ (same preparation) and vitamin D₂ (Drisdol). Oral alkalis were administered to control acidosis. Autopsy disclosed: chronic hydronephrotic pyelonephritis; calcification of the heart and the systemic arteries; early renal rickets in the ribs, the vertebrae and a humerus; lobular pneumonia; hemosiderosis of the spleen and the liver.

The second case of Andersen and Schlesinger⁵³ was that of a 6 month old boy who was admitted with a history of convulsions of one day's duration. He showed a diffuse papular cutaneous rash, signs of tetany and a round mass in the left flank. The hemoglobin content was 6.6 to 12 Gm. per hundred cubic centimeters; the erythrocyte count was 3,410,000 and the leukocyte count was 34,800, per cubic millimeter, with 83 per cent segmented neutrophils. Serum calcium was 6.5 to 9 mg., serum phosphorus 6.4 to 11.3 mg., serum phosphatase 11.4 Bodansky units, blood nonprotein nitrogen 68.4 to 125 mg., blood urea nitrogen 96.1 mg., serum albumin 4.4 Gm., serum globulin 3.1 Gm. and blood cholesterol 177 mg. per hundred cubic centimeters; the carbon dioxide-combining power of the blood was 16 to 41.4 volumes per cent. There was albuminuria, and *Staphylococcus aureus* and *Staph. albus* were present in the urine. Halisteresis of the long bones was shown

53. Andersen, D. H., and Schlesinger, E. R.: *Am. J. Dis. Child.* **63**:102, 1942.

by roentgenogram. Terminally, there was necrosis of the left buttock at the site of injections of calcium gluconate, and the blood pressure was 136 to 150 systolic and 90 diastolic. Calcium chloride, calcium gluconate and 300,000 units of vitamin D as 7-dehydrotachysterol alleviated the tetanus on admission. The patient also received 15 drops of a vitamin D preparation for two months of his hospital stay and calcium gluconate and sodium lactate orally for the last three weeks of life. Autopsy disclosed: chronic hydronephrotic pyelonephritis of the right kidney; a polycystic left kidney; calcification of the heart, the systemic arteries and the right kidney; pulmonary edema; hemosiderosis of the spleen.

Summary.—Of the 23 patients with chronic renal disease and metastatic calcification, the sex was male in 14 and female in 9. Their ages were between 6 months and 55 years, the disease occurring in 17 before the age of 40 and in only 1 who was over 50 years of age. Four infants were affected. The urine showed varying degrees of albuminuria,⁵⁴ with specific gravity ranging from 1.000 to 1.017, but generally being less than 1.010. When recorded,⁵⁵ the number of leukocytes and erythrocytes found in the urine was variable. The excretion of phenolsulfonphthalein⁵⁶ ranged from none to less than 10 per cent in two hours. Urea clearance⁵⁷ varied from less than 1 to 18 per cent. The level of hemoglobin and of blood erythrocytes was moderately to greatly depressed in several patients.⁵⁸ The level of serum calcium was depressed (6.7 mg. per hundred cubic centimeters),⁴³ depressed or normal (7.4 to 11.0 mg. and 6.5 to 9.0 mg.),⁵³ normal (9.3 to 11.0 mg.)⁵⁹ or elevated (11.7 to 13.6 mg.).⁶⁰ Serum inorganic phosphorus was elevated (5 to 16 mg. per hundred cubic

54. Butler.³⁹ Lightwood.⁴¹ Albright and others.⁴² Platt and Owen.⁴³ Smyth and Goldman.⁴⁴ Pollack and Siegal.⁴⁷ Price and Davie.⁴⁸ Castleman and Mallory.⁴⁹ Brown and Ginsburg.⁵¹ Herbert and others.⁵² Andersen and Schlesinger.⁵³

55. Butler.³⁹ Lightwood.⁴¹ Albright and others.⁴² Smyth and Goldman.⁴⁴ Shelling and Remsen.⁴⁵ Pollack and Siegal.⁴⁷ Castleman and Mallory.⁴⁹ Herbert and others.⁵² Andersen and Schlesinger.⁵³

56. Butler.³⁹ Smyth and Goldman.⁴⁴ Shelling and Remsen.⁴⁵ Pollack and Siegal.⁴⁷ Brown and Ginsburg.⁵¹

57. Lightwood.⁴¹ Smyth and Goldman.⁴⁴ Price and Davie.⁴⁸ Brown and Ginsburg.⁵¹

58. Albright and others.⁴² Platt and Owen.⁴³ Smyth and Goldman.⁴⁴ Shelling and Remsen.⁴⁵ Magnus and Scott.⁴⁶ Pollack and Siegal.⁴⁷ Castleman and Mallory.⁴⁹ Pons and Pappenheimer.⁵⁰ Brown and Ginsberg.⁵¹ Herbert and others.⁵² Andersen and Schlesinger.⁵³

59. Lightwood.⁴¹ Shelling and Remsen.⁴⁵ Magnus and Scott.⁴⁶ Castleman and Mallory.⁴⁹

60. Hubbard and Wentworth.³⁸ Smyth and Goldman.⁴⁴ Pollack and Siegal.⁴⁷ Price and Davie.⁴⁸ Brown and Ginsburg.⁵¹ Herbert and others.⁵²

centimeters) in 11 patients,⁶¹ although 2 also showed normal values on occasion. The alkaline phosphatase of the serum was slightly to moderately raised in 7 patients⁶² and normal in 2.⁵³ The blood non-protein nitrogen (45 to 364 mg. per hundred cubic centimeters)⁶³ and urea (143 to 560 mg. per hundred cubic centimeters)⁶⁴ or urea nitrogen (38.7 to 205 mg. per hundred cubic centimeters)⁶⁵ were increased moderately to greatly in all cases in which the values were recorded. The carbon dioxide-combining power of the blood was slightly to severely lowered (16 to 49.6 volumes per cent) in 7 patients.⁶⁶ The serum proteins (4.7 to 7.5 Gm. per hundred cubic centimeters) were somewhat lowered or normal. Blood cholesterol (177 to 210 mg. per hundred cubic centimeters) was slightly to moderately elevated in 6 patients.⁶⁷ Blood chlorides were depressed or normal in 4 patients.⁶⁸

The types of nephropathy found associated with metastatic calcification in the 23 cases of renal disease included chronic nephritis in 9, chronic hydronephrotic pyelonephritis in 4, chronic glomerulonephritis and chronic interstitial nephritis in 3 each, chronic suppurative hydronephrotic pyelonephritis (pyonephrosis) in 2 and subacute glomerulonephritis and active degenerative nephritis in 1 each. In 1 case,⁴⁰ amyloidosis involved the right kidney, and chronic suppurative hydronephrotic pyelonephritis affected the left kidney. In the second case of Andersen and Schlesinger,⁵³ chronic hydronephrotic pyelonephritis was present in the right kidney, and a polycystic left kidney was found. In the other 21 cases the lesion was bilateral as indicated.

The parathyroid glands of 15 patients were described. In 11 instances these glands were enlarged and showed diffuse hyperplasia of chief cells, characteristic of chronic renal insufficiency.⁴⁹ Five glands were found involved by Smyth and Goldman⁴⁴; 4 each by Shelling and Remsen,⁴⁵ Price and Davie,⁴⁸ Castleman and Mallory,⁴⁹ Pons and Pappenheimer⁵⁰,

61. Lightwood.⁴¹ Platt and Owen.⁴³ Smyth and Goldman.⁴⁴ Shelling and Remsen.⁴⁵ Pollack and Siegal.⁴⁷ Price and Davie.⁴⁸ Castleman and Mallory.⁴⁹ Brown and Ginsburg.⁵¹ Herbert and others.⁵² Andersen and Schlesinger.⁵³

62. Smyth and Goldman.⁴⁴ Shelling and Remsen.⁴⁵ Pollack and Siegal.⁴⁷ Price and Davie.⁴⁸ Castleman and Mallory.⁴⁹ Brown and Ginsberg.⁵¹ Herbert and others.⁵²

63. Platt and Owen.⁴³ Smyth and Goldman.⁴⁴ Shelling and Remsen.⁴⁵ Castleman and Mallory.⁴⁹ Pons and Pappenheimer.⁵⁰ Andersen and Schlesinger.⁵³

64. Butler.³⁹ Lightwood.⁴¹ Price and Davie.⁴⁸ Herbert and others.⁵²

65. Albright and others.⁴² Platt and Owen.⁴³ Pollack and Siegal.⁴⁷ Pons and Pappenheimer.⁵⁰ Brown and Ginsberg.⁵¹ Andersen and Schlesinger.⁵³

66. Smyth and Goldman.⁴⁴ Shelling and Remsen.⁴⁵ Pollack and Siegal.⁴⁷ Castleman and Mallory.⁴⁹ Brown and Ginsberg.⁵¹ Andersen and Schlesinger.⁵³

67. Lightwood.⁴¹ Platt and Owen.⁴³ Shelling and Remsen.⁴⁵ Brown and Ginsberg.⁵¹ Andersen and Schlesinger.⁵³

68. Smyth and Goldman.⁴⁴ Shelling and Remsen.⁴⁵ Castleman and Mallory.⁴⁹ Andersen and Schlesinger.⁵³

(first case), Herbert, Miller and Richardson⁵² and Andersen and Schlesinger⁵³ (first case); 3, by Magnus and Scott⁴⁶; 2, by Hubbard and Wentworth.³⁸ Pollack and Siegel⁴⁷ did not specify the number of enlarged glands present. In the second case of Andersen and Schlesinger⁵³ the left lower gland was affected. Lightwood⁴¹ described one normal gland in his case. Albright, Baird, Cope and Bloomberg⁴² mentioned enlarged lower parathyroid glands in their case, and Platt and Owen⁴³ stated that their patient's glands were not enlarged, but in neither instance was a microscopic description given. In the report of their second case Pons and Pappenheimer⁵⁰ did not mention the number or the histologic character of the enlarged parathyroid glands.

In the 13 cases in which the bones (including the ribs, the vertebrae, the long bones and the skull bones) were examined, osteitis fibrosa cystica was the lesion in 7, rickets in 4 and osteoporosis in 2.

Also noteworthy in 5 cases⁶⁹ was the use of vitamin D.

Calcium deposits were observed in the lungs in 12 cases, most often in the walls of the alveoli and in the intima and the media of arteries and veins, frequently in relation to the elastic tissue in these structures. Bronchi, bronchioles, alveolar ducts and lumens, capillaries and stroma were less commonly affected.

In 10 cases significant calcification was found in the kidneys, most often in the cells and lumens of convoluted and collecting tubules and in the stroma. Glomeruli and the basement membranes of tubules were less frequently involved. In only 1 instance was deposition of calcium described⁴⁴ in the smaller arteries and arterioles of the kidneys. In no case was calcification of renal veins shown.

In 11 cases calcification of the heart was described. The muscle fibers, chiefly those of the left ventricle, the stroma of the myocardium and the endocardium of the left auricle and ventricle were especially affected. Small arteries, capillaries, the mitral valve and the epicardium were involved to a minor degree.

In 15 cases the systemic arteries showed calcific changes. In addition to the aorta, the coronary, mesenteric, splenic, hepatic, renal, gastric, pancreatic, adrenal, uterine, vesical, hypogastric, thyroid, intercostal, vertebral, subclavian, axillary, brachial, iliac, femoral, popliteal, tibial and pedal arteries were affected. The calcium salts were found chiefly in the internal elastic lamella of the intima and in the media, often in relation to elastic fibrils.

In 3 cases, calcification involved the stomach; it was abundant in the interglandular stroma, but was also found in the basement membranes of the glands.

69. Lightwood.⁴¹ Pollack and Siegel.⁴⁷ Brown and Ginsberg.⁵¹ Andersen and Schlesinger.⁵³

Miscellaneous sites of calcification included periarticular structures,⁷⁰ dura,⁷¹ tentorium,⁴¹ trachea,⁴¹ parietal pleura,⁴¹ jejunum,³⁸ greater omentum,³⁸ splenic veins,³⁷ skin⁴³ and subcutaneous tissue.⁴⁶

Schmidt³⁷ found calcium phosphate and little calcium carbonate in the heart of his patient. As the oxide, calcium amounted to 2.3 per cent in the left ventricle and 0.6 per cent in the right ventricle of this patient by quantitative analysis. In their case, Smyth and Goldman⁴⁴ found calcium phosphate in the periarticular deposits and 20.92 mg. of calcium and 11.54 mg. of phosphorus by dry weight in the long bones. The calcium content of the kidneys of the second patient of Pons and Pappenheimer⁵⁰ was 1,300 mg. per hundred grams of wet tissue, compared with a normal of 11 mg. per hundred grams.

METASTATIC CALCIFICATION AND PRIMARY NEOPLASMS OF THE PARATHYROID GLANDS

The first case reported by Meyer⁷² concerned a married woman 43 years old, who had a therapeutic abortion for "renal inflammation" ten years before, a stillbirth nine years before, two spontaneous abortions eight years before and treatment for renal calculi for eight years. On the day of admission she fractured her left femur, which was amputated proximal to the cystic fracture site several weeks later. Osteitis fibrosa cystica of the bones of the lower extremities, absence of the left lower extremity, fracture of the right femur, calcification of the kidneys, a polyp of the uterine cervix and a myxoma of the right fallopian tube were demonstrated at autopsy. Although the organs of the neck were not examined, the patient presumably had a parathyroid neoplasm, for the changes in the bones and the kidneys were like those to be described in the summaries of other cases in this section.

Meyer's second patient was a man 36 years old, who had ataxia, severe pain in the extremities, atrophy of the thigh muscles, edema of the legs, hydrarthrosis of the left knee and polydipsia when he was first hospitalized. During the next two years he became gradually more disabled. On the day of his second admission he sustained fractures of the femurs, which were splinted. He later displayed general irritability, pallor, the typical changes of bone softening, fractures of the humeri and terminal bronchopneumonia. Autopsy disclosed: chief cell adenoma of the right inferior parathyroid gland; osteitis fibrosa cystica of the skull, vertebrae, ribs and long bones; multiple cysts and fractures of the long bones; calcification of the kidneys; right-sided bronchopneumonia; fatty metamorphosis of the liver.

70. Hubbard and Wentworth.³⁸ Smyth and Goldman.⁴⁴ Castleman and Mallory.⁴⁹ Herbert and others.⁵²

71. Lightwood.⁴¹ Smyth and Goldman.⁴⁴

72. Meyer, O.: *Frankfurt. Ztschr. f. Path.* 20:115, 1917.

Dawson and Struthers⁷³ reported the case of a man 49 years old, who fractured the lower third of his left humerus four months before, for which routine treatment was instituted. He displayed wasting and limitation of motion of the left upper extremity. Decalcification of the humeral fragments and an enlarged, decalcified skull were shown by roentgenogram. The urine revealed transient slight albuminuria, and biopsy of the left humerus revealed changes consistent with osteitis fibrosa cystica. About fourteen weeks later he sustained a second fracture of the left humerus proximal to the first fracture, as shown by roentgenogram; it was healing slowly. Over five months after the second fracture he was seized with a "heart attack" and died in less than a day. Autopsy disclosed: bronchopneumonia; a transitional chief cell adenoma of the left inferior parathyroid gland; osteitis fibrosa cystica of the left humerus and femur, the ribs and the skull; calcification of the heart, the lungs, the systemic arteries, the stomach, the kidneys, the spleen, the liver, the skeletal muscle, the dura and the pituitary and pineal glands.

Hoffheinz⁷⁴ described a 42 year old woman whose right arm had been fractured two years before and left knee joint one year before. Severe pains in the thighs and inability to walk confined her to bed for four months. Examination showed: atrophic thigh muscles; contractures of the adductor muscles of the thighs; limited motion of the joints; severe halisteresis of the skeleton, thick skull and long bones, skeletal cysts and two renal calculi on the right side by roentgenogram; a blood pressure of 85 systolic and 55 diastolic; blood nonprotein nitrogen 221 mg. per hundred cubic centimeters. Terminally there was uremic coma. Autopsy disclosed: diffuse hyperplasia of wasserhelle cells of four enlarged parathyroid glands; osteitis fibrosa cystica of skull, vertebrae and femurs; a fracture of the neck of the right femur; a cyst of the shaft of the left femur; chronic hemorrhagic suppurative cystoureteropyelonephritis; calcification of the kidneys, a coronary artery, the lungs and the stomach; renal calculi; fatty metamorphosis of the liver; nodular cortical hyperplasia of the adrenal glands.

Penecke⁷⁵ examined 2 patients at autopsy. The first was a man 38 years old, with a 16 Gm. transitional oxyphilic cell adenoma of the left inferior parathyroid gland, osteitis fibrosa cystica of the bones, and calcification of the contracted kidneys, the heart, the systemic arteries, the spleen, the tongue, the thyroid gland, the wall of the parathyroid neoplasm and the skin. The second was a woman 59 years old, with a 5 Gm. chief cell adenoma of the right inferior parathyroid gland, osteitis fibrosa cystica of the bones and calcification of the kidneys.

73. Dawson, J. W., and Struthers, J. W.: *Edinburgh M. J.* **30**:421, 1923.

74. Hoffheinz: *Virchows Arch. f. path. Anat.* **256**:705, 1925.

75. Penecke: *Centralbl. f. allg. Path. u. path. Anat.* **37**:535, 1926.

Fontana ⁷⁶ reported the case of a woman 26 years old, who had severe headache, loss of weight, fatigue and intermittent fever with vomiting and delirium for nine years before admission. In the year before she died she had albuminuria, edema of the ankles, vomiting, exertional dyspnea, weakness, fever, painful, fluctuant swellings adjacent to the large joints of the trunk and extremities and, terminally anasarca, hard peripheral arteries, increased dyspnea, precordial pain, diffuse pulmonary congestion, prostration and subnormal temperature. At autopsy the following conditions were demonstrated: a chief cell adenoma of the left superior parathyroid gland; osteomalacia of the skull, the sternum and the ribs; calcification of the kidneys, the heart, the systemic arteries, the lungs, the bursas and the dura; arteriolosclerosis of the spleen; fatty metamorphosis of the liver; atrophy of the ovaries; partial atrophy of the thyroid gland, the anterior lobe of the pituitary gland and the cortices of the adrenal glands.

Ask-Upmark ⁷⁷ reported the case of a man 46 years old, who suffered with loss of weight, pain in the feet and the joints of the hip and the knee for nine months, insomnia, anorexia, and pain in the head, the spine and the extremities for three months, so that he became bedridden, and cough, vomiting and occasional hemoptysis for two weeks. Examination showed: a systolic blood pressure of 175; emaciation; atrophic pharyngitis; dental caries; hemorrhagic gingivitis; pain on motion of the left hip and knee; a hemoglobin content of 76 per cent; erythrocytes 3,980,000 per cubic millimeter; leukocytes 14,200 per cubic millimeter, with 55 per cent neutrophils, 32 per cent lymphocytes and 13 per cent monocytes; serum calcium 10.1 mg., and blood nonprotein nitrogen 50 mg. per hundred cubic centimeters; slight albuminuria; lumbar lordosis, mottled lumbar vertebrae, halisteresis of the sacrum, the pelvic bones, the femurs, the scapulas, the humeri, the clavicles and the ribs and destruction of the cortex of the left femur, by roentgenogram; spontaneous fractures of the humeri and a femur; hard peripheral arteries. Terminally there were fecal and urinary incontinence, decubital ulcers and intermittent fever. Autopsy showed: a chief cell adenoma of a left parathyroid gland; osteitis fibrosa cystica of the skull, the vertebrae, the pelvic bones and the femurs; fractures of the humeri and the femurs; calcification of the kidneys and the coronary arteries; bronchopneumonia.

A woman 57 years old, described by Bergstrand,⁷⁸ had general weakness, severe constipation, piercing left-sided headaches localizing in the left ear, a systolic blood pressure of 190 to 205, and loss of 15 Kg. for seven months, and abdominal distention and severe exertional palpitation and dyspnea for two months, so that she was forced to go to bed. She

76. Fontana, A.: *Endocrinol. e pat. costit.* 4:401, 1929.

77. Ask-Upmark, E.: *Acta med. Scandinav.* 74:284, 1930.

78. Bergstrand, H.: *Acta med. Scandinav.* 76:128. 1931.

exhibited pallor, nervousness, a fine tremor of the fingers and a goiter of moderate size. Examination showed: blood pressure varying from 140 systolic and 95 diastolic to 180 systolic and 110 diastolic; a soft blowing systolic murmur over both the heart and the root of the aorta; a uterine tumor; a hemoglobin content of 95 per cent; erythrocytes 3,900,000 per cubic millimeter; leukocytes 4,800 per cubic millimeter, with segmented neutrophils 52 per cent, lymphocytes 36 per cent and monocytes 10 per cent; blood nonprotein nitrogen 40 mg. per hundred cubic centimeters; a basal metabolic rate of $+14$ to 41 per cent; total gastric acidity 22. Two years after discharge she had essentially the same symptoms and signs except for 35 per cent segmented neutrophils and 61 per cent lymphocytes in the blood, slight albuminuria and urinary specific gravity of 1.005 to 1.008, a total gastric acidity of 7, and widening of the right side of the superior mediastinum, seen by roentgenogram. Terminally a brown color of the skin developed, with fever, nausea, vomiting, and a painful mass in the right upper abdominal quadrant. At autopsy the following conditions were found: diffuse follicular hyperplasia of four enlarged parathyroid glands; osteitis fibrosa cystica of the skull, the lumbar vertebrae and the femurs; calcification of the kidneys, the renal and splenic arteries and the liver; cardiac hypertrophy; acute and chronic cholecystitis; cholelithiasis; mild diffuse hyperplasia of the thyroid gland; a leiomyoma of the uterus.

Paul⁷⁹ reported the case of a 56 year old man, who had anorexia, weakness and loss of weight for two years, polyuria and nocturia for one year, vomiting for one month and severe headache for one week. His blood pressure was 184 systolic and 100 diastolic, and he had albuminuria with many hyaline casts, frequent emesis, rapid pulse, an apical diastolic murmur and terminal acute cardiac failure. Autopsy disclosed: diffuse hyperplasia of wasserhelle cells of the two enlarged superior parathyroid glands; osteitis fibrosa cystica of the skull, the vertebrae, the ribs, the pelvic bones and the femurs; fractures of four ribs; cysts of the femurs; calcification of the kidneys, the thyroid arteries, the splenic arterioles and the liver; pulmonary edema; suppurative bronchitis; nodular cortical hyperplasia of the adrenal glands.

Hand⁸⁰ recorded the case of a man 39 years old, who had not felt well for nine years before admission. He had nocturia for four months, an attack of rheumatism two months before, penile pain, urgency and frequency for one month and milky penile discharge and loss of 30 pounds (13.6 Kg.) for two weeks. He displayed emaciation, pallor and a dry skin. Examination showed: a small thyroid gland; a pulse rate of 140 beats per minute; a blood pressure of 130 systolic and 70 diastolic; normal heart and lungs; a palpable right kidney; a small,

79. Paul, F.: Beitr. z. path. Anat. u. z. allg. Path. 87:503, 1931.

80. Hand, J. R.: S. Clin. North America 13:1365, 1933.

tender prostate; normal testes; albuminuria; erythrocytes, leukocytes and gram-negative intracellular diplococci in the urine; a hemoglobin content of 94 per cent; erythrocytes 4,990,000 per cubic millimeter; leukocytes 10,000 per cubic millimeter with neutrophils 49 per cent and lymphocytes 47 per cent; a sedimentation rate of 18 mm. in forty-five minutes; serum calcium 14.6 mg. and blood urea 46 mg. per hundred cubic centimeters; a phenolsulfonphthalein test with excretion of 56 per cent in two hours; a dilated colon and lumbar scoliosis by roentgenogram; severe cystitis by cystoscopy. He received a high caloric, high vitamin diet, a 5 per cent solution of dextrose intravenously, 8 drachms (31 Gm.) of citrocarbonate, 3 drachms (11.65 Gm.) of calcium lactate and 10 drops of viosterol daily. Between seven weeks and the time of his death, sixteen weeks later, he suffered successively from urinary frequency and loss of strength, scleritis, conjunctivitis, left corneal ulcer, low grade fever, tachycardia, pigmentation of the skin, atrophy of muscles, bloating and, terminally, from expectoration of much purulent sputum. Autopsy disclosed: a chief cell adenoma of the left inferior parathyroid gland; general osteoporosis of the skull and the vertebrae; subacute interstitial nephritis; multiple renal abscesses; calcification of the kidneys, the heart, and the lungs; hypertrophy of the urinary bladder; cystitis cystica; chronic prostatitis.

Khurgina⁸¹ reported the case of a woman 34 years old, who showed at autopsy severe gastritis and abdominal dermatitis, an adenoma of a parathyroid gland, osteosclerosis of the skeleton and calcification of the kidneys, the heart, the lungs and the stomach.

Laubmann⁸² described a case in which clinically there were observed osteitis fibrosa cystica, cutaneous calcium deposits, rigid arteries in the lower extremities, increased serum calcium, leukocytosis and terminal anemia. Autopsy revealed an adenoma of a parathyroid gland, osteitis fibrosa cystica of the bones, and calcium deposits in the heart, the blood vessels, the lungs, the spleen and the skin.

The 49 year old woman described by Hanes⁸³ had diagnoses of renal calcification and hydronephrosis of the right kidney on the basis of urinary, hematologic and roentgenologic studies five years before admission to the hospital. She had right infrascapular pain for twenty-one months, pain along the anterior aspect of the right third rib for seventeen months, and weakness, loss of weight and confinement to bed for three months. Examination showed: a temperature of 38.2 C. (100.7 F.); a pulse rate of 120 and a respiratory rate of 22 per minute; a blood pressure of 148 systolic and 90 diastolic; emaciation; a 2 cm. nodule at the lower pole of the left lobe of the thyroid gland; slight

81. Khurgina, P. A.: *Klin. med.* **11**:1238, 1933.

82. Laubmann, W.: *Verhandl. d. deutsch. path. Gesellsch.* **27**:229, 1934.

83. Hanes, F. M.: *Am. J. M. Sc.* **197**:85, 1939.

albuminuria; a hemoglobin content of 10 Gm. per hundred cubic centimeters; erythrocytes 3,500,000 and leukocytes 6,200 per cubic millimeter; serum calcium 20 to 22 mg., serum phosphorus 4.8 mg., phosphatase 23 Bodansky units, plasma nonprotein nitrogen 58 mg., and total serum protein 6.2 Gm. per hundred cubic centimeters; kidneys mottled by calcium deposits and bones decalcified, by roentgenogram; a phenolsulfonphthalein test with excretion of 40 per cent in ninety minutes. Terminally there were fever, tachycardia, weakness, nervousness, hoarseness, generalized pain, dyspnea and cyanosis. Autopsy disclosed: an adenoma of the left inferior parathyroid gland, chronic tubular and glomerular nephritis, and calcification of the kidneys, the heart, the arteries, the lungs and the stomach.

Oliver's⁸⁴ first case concerned a woman aged 57, who lost weight and strength for six months, had nausea, anorexia, vomiting and constipation for two weeks and intermittent drowsiness for two days before admission to the hospital. Examination showed: a temperature of 97 F.; a pulse rate of 88 and a respiratory rate of 15 per minute; a blood pressure of 160 systolic and 100 diastolic; a dry, brown tongue; a normal heart; emphysematous lungs; a palpable, tender left kidney; increased reflexes on the right side; albuminuria; leukocytes and hyaline casts in the urine; blood urea 76 to 136 mg. and serum calcium 17.4 mg. per hundred cubic centimeters; carbon dioxide-combining power 75.8 volumes per cent. Terminally, severe stupor, a blood pressure of 70 systolic and 50 diastolic, a dry skin and elevated vital signs were observed. Necropsy revealed: a chief cell adenoma of the right inferior parathyroid gland; calcium deposits in the kidneys, the heart, the systemic arteries, the lungs, the stomach and the liver; a small meningioma in the right side of the anterior cranial fossa.

Oliver's⁸⁴ second patient was a woman of 56 years who had two attacks of vomiting, anorexia, constipation and severe thoracic and abdominal pain one year before and three months before admission to the hospital. She displayed drowsiness, frequent vomiting, pallor and a dry skin and tongue. Examination showed: a temperature of 98.4 F.; a pulse rate of 100 and a respiratory rate of 10 to 35 per minute; a blood pressure of 104 systolic and 86 diastolic; a small tumor adjacent to the left side of the thyroid gland; feeble cardiac tones; basal rales in the lungs; a tender abdomen; depressed reflexes; albuminuria; a hemoglobin content of 61 per cent; erythrocytes 4,660,000 per cubic millimeter; blood urea 176 mg. per hundred cubic centimeters, carbon dioxide-combining power 50.7 volumes per cent. Terminally she had uremia. Necropsy demonstrated a chief cell adenoma of the left inferior parathyroid gland and calcification of the kidneys, the heart, the systemic arteries, the lungs and the stomach.

84. Oliver, W. A.: *Lancet* 2:240, 1939.

Smith and Cooke⁸⁵ reported the case of a woman 44 years old, who suffered from attacks of dizziness, rheumatism of one hand, limp, bowing of the thighs, severe pain in the lower extremities and constipation during the thirty months before she was admitted to the hospital. She had abdominal pain, vomiting, constipation and frequency of urination. Examination showed: albuminuria; serum calcium 23 mg. and blood urea 155 mg. per hundred cubic centimeters. Terminally there were sphincter incontinence, delirium, tachycardia, hyperpnea, nausea and abdominal distention. Autopsy disclosed: a chief cell adenoma of the right inferior parathyroid gland; osteitis fibrosa cystica of the skull, one ilium and one femur; calcification of the kidneys; pancreatic fat necrosis.

Alexander and co-workers⁸⁶ described a 29 year old woman who experienced weakness, anorexia, fatigability, loss of weight and vomiting during the twenty-one months before she was admitted to the hospital. The hemoglobin content was 12.5 Gm. per hundred cubic centimeters; the erythrocytes numbered 4,350,000 per cubic millimeter and the sedimentation rate was 62 mm. in an hour. There was tachycardia, and terminally there were abdominal pain, restlessness and cyanosis. Necropsy revealed a chief cell adenoma of the right inferior parathyroid gland, osteitis fibrosa cystica of the skull, the ribs and the vertebrae and calcification of the kidneys and the lungs.

Cope⁸⁷ reported the case of a man of 68 years who had weakness, especially of the lower extremities, and eventual confinement to bed during the eighteen months before hospitalization. Examination showed: transient albuminuria; a phenolsulfonphthalein test with excretion of 12 per cent in two hours; serum calcium 10 to 11.3 mg., serum phosphorus 4.7 to 5.6 mg., serum phosphatase 19 to 22.5 units, total serum protein 6.4 Gm., blood nonprotein nitrogen 29 mg. and blood uric acid 6.6 mg. per hundred cubic centimeters; plasma chlorides 107 to 115 milliequivalents per liter; carbon dioxide 16 to 24 milliequivalents per liter; rarefied areas in the skull, decalcified spine, pelvis and long bones, and cysts in the second lumbar vertebra and one ilium, by roentgenogram; later normal values for serum calcium, phosphorus and phosphatase. There were several episodes of hematemesis, and terminally signs of pyelonephritis. Autopsy demonstrated: an adenoma of the right superior parathyroid gland; osteitis fibrosa cystica of the skull, the vertebrae, the ribs and one ilium; calcification of the lungs; two tumors of the stomach, diagnosed leiomyoma.

Anderson⁸⁸ recorded the calcific and other pathologic changes observed only in the kidneys of a woman of 47 years who had operative

85. Smith, F. B., and Cooke, R. T.: *Lancet* 2:650, 1940.

86. Alexander, H. B.; Pemberton, J. de J.; Kepler E. J., and Broders. A. C.: *Am. J. Surg.* 55:157, 1944.

87. Cope, O.: *Surgery* 16:273, 1944.

(Footnotes continued on next page)

removal of a transitional oxyphilic cell adenoma of the right superior parathyroid gland and died several months later.

Gissel and Bufe⁸⁹ described a 27 year old woman who clinically showed osteitis fibrosa cystica and an enlarged thyroid gland and whose serum calcium was determined to be 8.4 to 9.1 mg. per hundred cubic centimeters. Operative exploration of the neck revealed four grossly normal parathyroid glands, which were removed along with two thirds of the thyroid gland and a persistent thymus. Two of the parathyroid glands were transplanted into a rectus muscle. For twenty-two days after operation the patient received supplementary calcium and 60 cc. of dihydrotachysterol. Her blood calcium rose from 6.5 to 18 mg. per hundred cubic centimeters in this period, near the end of which weakness developed, with loss of weight, vomiting and severe headaches, and the patient died. Osteitis fibrosa cystica of the skeletal bones and calcification of the kidneys, the heart, the lungs, the liver and the pancreas were observed at autopsy. Although no histologic description of the parathyroid glands was given and no definite parathyroid neoplasm was found, the changes in the bones and the kidneys strongly indicated that such a tumor was present.

Summary.—There were 21 patients with primary parathyroid neoplasm associated with metastatic calcification. Of 20 of these the sex was male in 7 and female in 13. In 20 cases the age varied between 26 and 68 years, the disease occurring before the age of 40 in 7 and after that age in 13. The urine showed varying amounts of albumin,⁹⁰ with specific gravity ranging from 1.005 to 1.018. The numbers of leukocytes and erythrocytes in the urine varied.⁹¹ The phenolsulfonphthalein excretion⁹² varied from 12 to 56 per cent in two hours. The levels of hemoglobin and erythrocytes were moderately lowered in several patients.⁹³ The values of serum calcium were normal (10.0 to 11.3 mg. per hundred cubic centimeters)⁹⁴ or moderately to tremendously elevated (12 to 23 mg).⁹¹ The values for serum inorganic phosphorus (4.7 to 5.6 mg. per hundred cubic centimeters) and phosphatase (19 to 23 Bodansky units) were recorded in only 2 cases.⁹⁵ The blood nonprotein nitrogen ranged between 29 and 221 mg. per hundred cubic centimeters

88. Anderson, W. A. D.: *Endocrinology* 24:372, 1939.

89. Gissel, H., and Bufe, W.: *Deutsche Ztschr. f. Chir.* 256:58, 1942.

90. Ask-Upmark.⁷⁷ Bergstrand.⁷⁸ Paul.⁷⁹ Hand.⁸⁰ Hanes.⁸³ Oliver.⁸⁴ Smith and Cooke.⁸⁵ Cope.⁸⁷

91. Hand.⁸⁰ Hanes.⁸³ Oliver.⁸⁴ Smith and Cooke.⁸⁵

92. Hand.⁸⁰ Hanes.⁸³ Cope.⁸⁷

93. Ask-Upmark.⁷⁷ Bergstrand.⁷⁸ Paul.⁷⁹ Hand.⁸⁰ Oliver.⁸⁴ Alexander and others.⁸⁶

94. Ask-Upmark.⁷⁷ Cope.⁸⁷

95. Hanes.⁸³ Cope.⁸⁷

in 5 patients,⁹⁶ and the blood urea between 46 and 176 mg. per hundred cubic centimeters in 4.⁹⁷ Other blood chemistry values were too fragmentary for consideration.

The neoplasm found in the parathyroid glands was chief cell adenoma in 10 cases, adenoma of unspecified type in 4, diffuse hyperplasia of wasserhelle cells in 3 and transitional oxyphilic cell adenoma in 2; in 2 cases a neoplasm was not demonstrated.

The lesion involving the bones in 17 cases was designated as osteitis fibrosa cystica in 14 cases and as osteomalacia, osteoporosis and osteosclerosis in 1 each. Of the 4 remaining cases, the bones were not examined in 2 and not described microscopically in 2. The bones examined the most frequently were the skull bones (11), the vertebrae (9), the femurs (7), the ribs (6) and the pelvic bones (5). Vitamin D was employed in the treatment of 2 patients.⁹⁸

Calcium deposits were found in the lungs in 12 cases, frequently in alveolar walls, bronchi and capillaries and occasionally in arteries, veins and stroma.

In 19 cases calcification affected the kidneys, involving mainly the stroma and the cells and lumens of the convoluted and collecting tubules. The glomeruli, the arterioles and the arteries were fairly frequent sites of calcium deposition.

In 9 cases, calcium deposits were observed in the heart, preponderantly in the muscle fibers of the left ventricle, but were sometimes present in the left auricle, the capillaries and the stroma.

In 10 cases the systemic arteries were involved by calcific changes, including the aorta and the coronary, thyroid, adrenal, renal, hepatic, gastric, ovarian, meningeal, brachial, radial, iliac and femoral arteries. The internal elastic lamina of the intima and the elastic fibrils of the media were the sites of calcium deposits.

In 6 cases the stomach was the site of calcification, which involved the interglandular stroma, gland cells and blood vessels.

Miscellaneous deposits of calcium were observed in the liver,⁹⁹ the spleen,¹⁰⁰ the skin,¹⁰¹ the dura,¹⁰² the thyroid gland,⁷⁵ the bursas,⁷⁶ the pancreas⁸⁹ and the tongue.⁷⁵

Chemical analyses of calcified tissues were lacking in all 21 cases.

96. Hoffheinz.⁷⁴ Ask-Upmark.⁷⁷ Bergstrand.⁷⁸ Hanes.⁸³ Cope.⁸⁷

97. Hand.⁸⁰ Oliver.⁸⁴ Smith and Cooke.⁸⁵

98. Hand.⁸⁰ Gissel and Bufo.⁸⁹

99. Dawson and Struthers.⁷³ Bergstrand.⁷⁸ Paul.⁷⁹ Oliver.⁸⁴ Gissel and Bufo.⁸⁹

100. Dawson and Struthers.⁷³ Penecke.⁷⁵ Laubmann.⁸²

101. Penecke.⁷⁵ Laubmann.⁸²

102. Dawson and Struthers.⁷³ Fontana.⁷⁶

METASTATIC CALCIFICATION AND HYPERVITAMINOSIS D

A summary of the cases of metastatic calcification associated with hypervitaminosis D should be prefaced by reference to cases of bone disease,²⁸ chronic renal disease⁶⁰ and primary neoplasm of the parathyroid glands,⁹⁸ in which vitamin D employed in therapy may have contributed somewhat to the metastatic calcification observed in them.

A 5½ month old boy, described by Putschar,¹⁰³ took 6 drops of a proprietary preparation of irradiated ergosterol in oil daily for fourteen weeks, suffered from loss of weight and vomiting for ten weeks and showed slight albuminuria, a thickened skin and periods of low temperature for four weeks before he died with a terminal high fever. Autopsy disclosed calcification of the kidneys, chronic myocarditis, moderate fatty metamorphosis of the liver, subcutaneous lipid granuloma, an accessory spleen and emaciation. The parathyroid glands and bones were not described.

Thatcher¹⁰⁴ reported the case of an 18 month old boy who was afflicted by poor gain in weight and dyspepsia for thirteen months before being admitted to the hospital. Five months before, he had an attack of diarrhea. For five months he received irradiated ergosterol. Examination showed: general weakness; anorexia; pallor; loss of weight; an open anterior fontanel; a nightly temperature of 99.6 F.; moderate albuminuria and ketonuria; blood urea 90 mg. per hundred cubic centimeters; a systolic blood pressure of 78; apathy; hyperpnea. The course was downhill in the last twelve days of life. Autopsy demonstrated calcification of the kidneys and severe fatty metamorphosis of the liver. The ribs were not remarkable. The parathyroid glands were not examined.

Thatcher's¹⁰⁵ second patient was a boy 11½ months old, who showed pallor, anorexia, nervousness and constipation for four months, during which he received cod liver oil, a little of a proprietary preparation of malt extract and bone marrow and two ultraviolet ray treatments. Examination revealed loss of weight, pallor, weakness, prostration, a low temperature, atrophy of muscles, slight albuminuria. Terminally there were severe pyrexia and convulsions. Calcification of the kidneys, slight fatty change of the liver, normal bones and one intact parathyroid gland were observed at necropsy.

The boy observed by Ross and Williams¹⁰⁶ was one of premature twins and 8 to 14 months old. He received irradiated ergosterol for several months, during which he had anorexia, loss of weight and vomiting; he died suddenly. Autopsy disclosed asphyxia due to aspira-

103. Putschar, W.: *Ztschr. f. Kinderh.* 48:269, 1929.

104. Thatcher, L.: *Edinburgh M. J.* 38:457, 1931.

105. Thatcher, L.: *Lancet* 1:20, 1936.

106. Ross, S. G., and Williams, W. E.: *Am. J. Dis. Child.* 58:1142, 1939.

tion of food, bronchopneumonia and calcification of the kidneys, the heart, the arteries, the lungs and the stomach. The bones and the parathyroid glands were not described.

Gissel and Bufe⁸⁹ described experiments with 4 infants afflicted with meningocele and hydrocephalus. The first infant received over 900 cc. of dihydrotachysterol in one hundred and twenty-eight days of life. The blood calcium rose from 6.6 mg. per hundred cubic centimeters of serum on the thirty-seventh day to 9.4 mg. on the sixty-ninth day. Calcification of the kidneys, ascending meningitis and pyocephalus were observed at necropsy. The second infant was given over 350 cc. of a proprietary preparation of irradiated ergosterol in oil and over 270 cc. of a proprietary high potency vitamin A preparation in fifty-four days of life. Calcification of the kidneys, ascending meningitis and internal hydrocephalus were demonstrated at autopsy. The bones and the parathyroid glands of both infants were not described. The other 2 infants got both substances for eleven and twenty-two days but had no calcium deposits in their viscera.

Wolf¹⁰⁷ described a 3 month old boy who was born with spina bifida, lumbosacral meningocele, paralysis of the lower extremities and an enlarged head. Between eight and 6 weeks before hospitalization, he had a temperature of 101 to 104 F., and the serum calcium was 11.5 mg. and the serum phosphorus 4.7 mg. per hundred cubic centimeters. During this time, he retained about 3,500,000 units of electrically activated ergosterol given in 300,000 unit daily doses, and he suffered from an infection of the scrotum due to coliform bacilli and streptococci. Over a period of six weeks, the scrotal infection subsided, the head increased in girth, and low temperatures and greatly elevated spinal fluid protein were observed. He died suddenly during a cisternal tap. Calcification of the kidneys, hydrocephalus, meningocele, spina bifida and marasmus were found at necropsy. No mention was made of the bones or of the parathyroid glands.

Bauer and Freyberg¹⁰⁸ reported the case of a woman 32 years old, who suffered during the three years before her first hospitalization from recurrent attacks of diarrhea, malaise and headache. She was again admitted three years after discharge, complaining that there had been headaches, nasal obstruction and postnasal drip in the interim. For two years she had a hacking, mildly productive cough. For one year she took 100,000 units of vitamin D daily. From six to three months before admission she took 500,000 units or more daily. For three months she had swollen legs and feet, nodules on the extensor tendons of the fingers, pressure areas on the elbows and buttocks, thickened palms, pains in the extremities, easy fatigue, anorexia and loss of weight. Exam-

107. Wolf, I. J.: *J. Pediat.* 22:707, 1943.

108. Bauer, J. M., and Freyberg, R. H.: *J. A. M. A.* 130:1208, 1946.

ination disclosed: resting dyspnea; restlessness; pallor; distended cervical veins; diastolic gallop rhythm and transient rough diastolic murmur over the base of the heart; masses over the buttocks and sacroiliac joints; thickened, knotty palms; slight albuminuria; a hemoglobin content of 54 per cent; leukocytes 10,600 to 17,800 per cubic millimeter, with segmented neutrophils 82 per cent and lymphocytes 16 per cent; a sedimentation rate of 45 mm. per hour; serum albumin 3.7 Gm. and serum globulin 2.1 Gm. per hundred cubic centimeters; increased density of the lower lobe of the right lung by roentgenogram. Terminally there were cough, lethargy, epigastric pain, emesis, low grade fever and early gangrene of the right leg and foot. Autopsy revealed: calcification of the kidneys, the heart, the arteries, the lungs, the dura, the joints and the subcutaneous tissue; chronic duodenal ulcer; acute gastric and duodenal ulcers; chronic interstitial pancreatitis; perivascular cerebral hemorrhages; new-formed bone in the ribs and the sternum. The parathyroid glands were not described.

A man 44 years old, described by Mulligan,¹⁰⁹ had been taking large daily doses of a vitamin D preparation and alkaline salts for six months before he was admitted to the hospital. For two weeks he suffered from weakness, restlessness, drowsiness and alternate stupor and delirium. Examination showed: coma; a temperature of 99.8 F.; labored respiration; pallor; a loud blowing systolic precordial murmur; intact lungs; albuminuria; many granular casts and erythrocytes in the urine; a diffuse fine mottling of the lungs, by roentgenogram. Terminally there were irregular tachycardia, cough productive of abundant mucoid sputum, and continued coma. Autopsy disclosed: calcification of the kidneys, the heart, the arteries, the lungs, the stomach and the pancreas; pulmonary edema; hypertrophy of the heart and the kidneys; mural thrombosis of the left auricle; thrombosis of the renal veins; infarcts of the spleen; acute focal pancreatitis; chronic cholecystitis; cholelithiasis; slight fatty metamorphosis of the liver; osteoclasia of a rib; aspermiogenesis. The parathyroid glands were not examined. Chemical analysis revealed calcium phosphate in the kidneys and in the stomach and calcium carbonate in the lungs.

Summary.—Of the 9 patients in whom metastatic calcification was associated with hypervitaminosis D, 7 were infants and 2 were adults. Of the 7 whose sex was stated, 6 were males and 1 was a female. Vitamin D in some form played a chief causal role, and the kidneys were calcified in all 9 cases. The kidneys grossly were normal in size or enlarged, pale, swollen and yellow. The heart, the arteries and the lungs were calcified in 3 cases and the stomach in 2. The bones were not examined in 5 cases, were normal in 2, showed osteoclasia in 1 and new-

109. Mulligan, R. M.: *Am. J. Path.* 22:1293, 1946.

formed bone in 1. A single parathyroid gland was normal in 1 case¹⁰⁵; in all the others the parathyroid glands were not described. In 1 case chemical analysis demonstrated calcium carbonate in the lungs and calcium phosphate in the kidneys and the stomach.

METASTATIC CALCIFICATION AND UNCERTAIN ETIOLOGIC FACTORS

A man 29 years old, described by Grohe,¹¹⁰ showed tuberculosis of the lungs, the meninges and the ileum, calcification of the colon, fibrosis of the liver, bronchitis, thinning of the calvarium and fibrous pleuritis at autopsy.

Chiari⁸ observed severe pyloric stenosis and calcification of the lungs and the kidneys in a female of unstated age. The bones showed no anomaly.

Hlava¹¹¹ recorded the case of a woman 42 years old, who had a large inguinal hernia on the left side for twenty-four years, dyspnea and dry cough for one year, and insanity for one month before admission to a hospital. Terminally she showed continued vomiting, severe dyspnea and diffuse cutaneous emphysema. In addition to these findings, she had strangulation of loops of intestine in the hernial sac, emphysema and calcification of the lungs, rupture and atelectasis of the right lung, pneumothorax on the right, pneumoperitoneum and hypertrophy of the right side of the heart. The skull was not grossly remarkable. A chemical test indicated deposition of calcium carbonate in the lungs.

A woman 42 years old, described by Kischensky,¹¹² suffered during life from obesity, epigastric pain and severe vomiting. Calcification of the lungs and the stomach, edema of the lungs, fatty degeneration of the heart, amyloidosis of the spleen, brown atrophy of the liver, chronic interstitial nephritis and fibrous pleuritis were observed at autopsy. No skeletal disease was demonstrated. Chemical analysis revealed calcium acid phosphate in the lungs.

At autopsy a man 36 years old, described by Hedinger,¹¹³ showed extensive calcification of viscera (heart, lungs, liver and kidneys), true contracted kidneys, widespread osteomalacia and cysts of the right humerus and the twelfth thoracic vertebra. The liver contained calcium phosphate by chemical test.

Liebscher¹¹⁴ recorded the case of a 26 year old woman who at autopsy was found to have calcification of the heart, the arteries, the spleen and the liver and tuberculosis of the lungs and of the bronchial

110. Grohe, F.: *Virchows Arch. f. path. Anat.* **13**:277, 1858.

111. Hlava, J.: *Wien. med. Bl.* **36**:1099 and 1165, 1882.

112. Kischensky, D.: *Centralbl. f. allg. Path. u. path. Anat.* **12**:674, 1901.

113. Hedinger, E.: *Cor.-Bl. f. schweiz. Aerzte* **39**:833, 1909.

114. Liebscher, cited by Hedinger.¹¹³

and cervical lymph nodes. The hepatic deposits consisted largely of calcium phosphate. The bones were not mentioned.

The case reported by Surbek¹¹⁵ was that of a girl born with a paretic right arm and dying of cardiac failure at the age of 2 days. Autopsy disclosed: calcification of the aorta, the systemic arteries, the lungs, the adrenal glands, the kidneys and the ovaries; chronic myocarditis, periarteritis, pancreatitis and myometritis; fatty metamorphosis of the liver; serofibrinous pericarditis. The humeri and the femurs were intact.

Harbitz¹¹⁶ recorded the case of a woman 41 years old, who had two attacks of acute rheumatism. Later she had, dyspnea, epigastric pain, hematemesis, epistaxis and menorrhagia for three years, cyanosis and swelling of the legs for several months, and orthopnea, enlarged abdomen and oliguria for seven weeks before she entered a hospital. Examination revealed: a pulse rate of 104 and a respiratory rate of 34 per minute; a blood pressure of 110 systolic; facial cyanosis; anasarca; clubbed fingers; cardiac enlargement; a systolic murmur loudest at the apex; an accentuated pulmonic second sound; prolonged respiratory expiration; hepatomegaly; ascites; blood erythrocytes 8,450,000 and leukocytes 11,600 per cubic millimeter. Terminally there was cardiac irregularity. Necropsy revealed: calcification and hypertrophy of the lungs; old tuberculosis of the lungs, the bronchial and cervical lymph nodes and the uterine tubes; hypertrophy of the right side of the heart; fibrous pleuritis; ascites. The bones and the parathyroid glands were not remarkable. Chemical analysis of the lungs showed 80 per cent calcium phosphate and 18 per cent calcium carbonate.

A boy described by Bross¹¹⁷ died twenty minutes after birth and showed calcification of the lungs and a closed ductus arteriosus at autopsy. Chemical test of the lungs proved the presence of calcium salts. The bones were not remarkable.

A 6 day old girl was observed by Marsden¹¹⁸ to have a generalized cutaneous rash of punched-out, crusted small ulcers, umbilical sepsis, a right scapular subcutaneous abscess, a temperature of 101 F., gradually progressive jaundice, manual and pedal spasm, paresis of the left side of the face, multiple hard subcutaneous nodules and cutaneous ecchymoses. In addition to these findings, autopsy revealed: calcification of the lungs, the liver, the adrenal glands, the kidneys, the thymus and the subcutaneous tissue; diffuse necrosis of the liver; tubular and glomerular nephritis; ulcers of the intestines; hemorrhages in the lungs, the adrenal glands and the kidneys; hemolytic streptococci in a left clavicular subcutaneous abscess and in the heart's blood; fibrous pleu-

115. Surbek, K.: *Centralbl. f. allg. Path. u. path. Anat.* **28**:25, 1917.

116. Harbitz, F.: *Arch. Int. Med.* **21**:139, 1918.

117. Bross, K.: *Centralbl. f. allg. Path. u. path. Anat.* **49**:229, 1930.

118. Marsden, J. P.: *Brit. J. Child. Dis.* **27**:193, 1930.

ritis, on the right side, and emaciation. Parathyroid gland tissue was abundant and active. A rib and a femur were normal.

The second patient of Grayzel and Lederer²⁷ was a married woman aged 25, who was delivered spontaneously at seven months of pregnancy, seven weeks before hospitalization. She had a dry cough for seven weeks, weakness, lassitude and vertigo for one month, and pyuria for two weeks. Examination showed: a temperature of 100.4 F.; a pulse rate of 120; a blood pressure of 124 systolic and 60 diastolic; a normal heart; decreased breath sounds over the lower lobe of the right lung; hepatomegaly; local tibial tenderness; hydrothorax on the right, by roentgenogram; albuminuria; a hemoglobin content of 53 per cent, erythrocytes 3,400,000 and leukocytes 6,700 to 24,400 per cubic millimeter, with stab neutrophils 4 to 20 per cent, segmented neutrophils 59 to 78 per cent and lymphocytes 12 to 28 per cent; serum calcium 15.6 mg., serum phosphorus 3.5 mg., serum phosphatase 8.2 units, serum albumin 2.3 Gm., serum globulin 2.4 Gm., blood urea 71 mg., blood uric acid 9.6 mg., blood cholesterol 154 mg., total lipids of blood 614 mg., plasma chlorides 316 mg. and serum sodium 280 mg. per hundred cubic centimeters; total base 140 milliequivalents; icterus index 3.6; carbon dioxide-combining power of blood 65.5 volumes per cent; a sedimentation rate of 210 mm. per hour; a myeloid-erythroid ratio of 80:20 in the bone marrow; nodules in the skin of the breasts, the axillas, the groins, the thighs and the right leg. Terminally there occurred fever (temperature of 105 F.), dyspnea, cyanosis, tachycardia, dependent edema, Cheyne-Stokes respiration and semistupor. Necropsy disclosed: calcification of the heart, the systemic arteries, the lungs, the liver, the skin and the larynx; infarcts of the spleen; chronic passive hyperemia of the liver; proctitis; cystitis; bilateral hydrothorax; a decubital ulcer over the right hip. The bones were not described. Three small parathyroid glands were microscopically normal. Chemical analysis of the cutaneous deposits showed 114 mg. of calcium and 1.6 mg. of phosphorus, compared with normal values of 0.1 mg. of calcium and 1.1 mg. of phosphorus.

An 8 week old girl, described by Baggenstoss and Keith,¹¹⁹ was admitted to a hospital after she had suffered for several hours from vomiting, belching and distended abdomen. Examination showed severe dehydration, soft fontanels, a coated tongue and cold extremities. She died four days later. Cardiac hypertrophy, fatty metamorphosis of the liver and calcification of the heart, the systemic arteries and the kidneys were found at autopsy. Other organs were not remarkable.

The sixth case reported by Virchow in his first paper,¹ the second case detailed in his second paper,⁴ the case of Babes¹²⁰ and Kaufman's

119. Baggenstoss, A. H., and Keith, H. M.: *J. Pediat.* 18:95, 1941.

120. Babes, V.: *Virchows Arch. f. path. Anat.* 105:511, 1886.

case^s have been excluded from this collection of genuine examples of metastatic calcification. As a matter of fact, a few instances of this condition included in the clinicopathologic abstracts of cases accepted by me might be questioned by more severe critics.

Summary.—No systematic summary of the heterogeneous group of cases in this section has been attempted. Of the 12 patients included, 4 were infants and 8 were adults. Three were males and 9 were females. In 9 cases no hint of the etiologic basis for the calcification was given by the authors¹²¹ who recorded them. Chronic interstitial nephritis may have been significant in causing calcification in the case reported by Kischensky,¹¹² but the evidence presented was too scanty for it to be classified specifically. The same was true for the case of Hedinger,¹¹³ in which true contracted kidneys, widespread osteomalacia and cysts of a humerus and a vertebra were demonstrated to suggest that a parathyroid neoplasm may have been present and not investigated. Also to be considered is the possibility that the calcific deposits in the lungs of Harbitz'¹¹⁶ patient represented dystrophic calcification on the basis of miliary tubercles. The parathyroid glands were not described in 8 cases¹²² and were not remarkable in 4 cases.¹²³ Chemical analysis revealed calcium carbonate in the lungs,¹²⁴ calcium phosphate in the lungs¹²⁵ and the liver¹²⁶ and calcium salts in the lungs¹¹⁷ and the skin.²⁷ Therapy with vitamin D or salts did not play a role in causing calcification in any of the 12 cases.

EXPERIMENTAL PRODUCTION OF METASTATIC CALCIFICATION

The reports of metastatic calcification produced by experimental use of extracts of parathyroid gland, vitamin D and minerals will be summarized, with preponderant emphasis on studies of the tissues of animals.

In dogs given injections of parathyroid extract, Hueper¹²⁷ observed psychic depression, vomiting, bradycardia, decreased blood coagulation time, hematemesis, melena, oliguria or anuria, slight albuminuria, weakness, dizziness, coma and death. At autopsy calcification involved occasional cardiac muscle fibers, a few elastic fibrils in the alveolar septums of the lungs, the gland cells of the fundic mucosa of the stomach, the circular muscle fibers of the duodenum, the basement membranes of

121. Grohe.¹¹⁰ Chiari.⁸ Hlava.¹¹¹ Liebscher.¹¹⁴ Surbek.¹¹⁵ Bross.¹¹⁷ Marsden.¹¹⁸ Baggenstoss and Keith.¹¹⁹ Grayzel and Lederer.²⁷

122. Grohe.¹¹⁰ Chiari.⁸ Hlava.¹¹¹ Kischensky.¹¹² Hedinger.¹¹³ Liebscher.¹¹⁴ Surbek.¹¹⁵ Bross.¹¹⁷

123. Harbitz.¹¹⁶ Marsden.¹¹⁸ Baggenstoss and Keith.¹¹⁹ Grayzel and Lederer.²⁷

124. Hlava.¹¹¹ Harbitz.¹¹⁶

125. Kischensky.¹¹² Harbitz.¹¹⁶

126. Hedinger.¹¹³ Liebscher.¹¹⁴

127. Hueper, W. C.: Arch. Path. 3:14, 1927.

the glomerular capsules and tubules, the cells of the tubules, the casts in the lumens of the tubules of the kidneys and the colloid and the stroma of the thyroid gland. Also observed were acute gastric and duodenal ulcers, necrotic cells in the centers of the hepatic lobules and in the renal tubules, and hemorrhages in the stomach, the duodenum and the brain.

In 2 dogs receiving 100 to 150 units of a parathyroid extract, Learner¹²⁸ found serum calcium levels of 16.8 and 19.6 mg. per hundred cubic centimeters. Calcification affected the stroma and a few fibers of the heart, the intima of the coronary and splenic arteries, the elastic fibrils of the alveolar septums and the bronchi of the lungs, the basement membrane of the gland crypts and the parietal cells of the stomach, the cells and the sinusoids at the centers of the hepatic lobules, and the epithelial cells, the basement membranes and the lumens of the tubules, the walls of the arteries and the loops of glomeruli of the kidneys.

Large doses of a parathyroid extract injected subcutaneously into young growing guinea pigs by Jaffe, Bodansky and Blair¹²⁹ caused metastatic calcification in the heart, the lungs, the gastric and intestinal mucosa, the kidneys and the subcutaneous tissue. Generalized bone lesions, prominent at the costochondral junctions, the metaphyses and the diaphyses, included resorption of bone, degeneration and fibrosis of marrow and cessation of bone formation at the zones of active growth in forty-eight hours. Four days after the last injection, extensive subperiosteal callus and osteoid tissue were found.

Chown, Lee and Teal¹³⁰ injected parathyroid extract intermittently, both subcutaneously and intraperitoneally, into two strains of newborn rats. One strain was albino and afflicted with severe hydronephrosis in 0.5 per cent and mild hydronephrosis in 4 per cent of the animals. The other strain was hooded and had normal viscera. Within the first few days, granular calcium was found in the straight tubules, in the adjacent stroma or in the lumens of tubules. In rats given injections for fifteen to fifty-two days, the peritubular calcium masses were lacking. The albino rats showed greater calcium deposits. The same authors¹³¹ reported on 147 rats given injections and 86 controls in longer experiments lasting up to one hundred and seventy-four days. Calcium did not involve the sclerotic glomeruli. In the first day the tubules and the pelves were dilated in relation to interstitial calcium deposits. In two days, tubular dilatation at the corticomedullary junction and peritubular fibrosis were noted. By thirteen days, some tubules were dilated and others collapsed within hyalinized basement membranes merging into the surrounding focally fibrotic, shrunken, chronically

128. Learner, A.: *J. Lab. & Clin. Med.* **14**:921, 1929.

129. Jaffe, H. L.; Bodansky, A., and Blair, J. E.: *Arch. Path.* **11**:207, 1931.

130. Chown, B.; Lee, M., and Teal, J.: *Canad. M. A. J.* **35**:513, 1936.

131. Chown, B.; Lee, M., and Teal, J.: *Canad. M. A. J.* **36**:7, 1937.

inflamed stroma. The calcium within the tubules increased with prolonged injection of the extract.

Cantarow, Stewart and Housel¹³² injected 2,700 to 3,500 units of parathyroid extract intramuscularly into 5 dogs, 4 of which were female and 1 male. The serum calcium ranged between 17.5 and 20.3 mg. and the total serum proteins between 4.4 and 6.5 Gm. per hundred cubic centimeters. Calcification involved the heart, the arteries, the stomach and the kidneys. One female was pregnant with 9 fetuses 140 to 150 mm. long and showing no calcific or degenerative changes. The calcium deposits in the myocardium, the parietal cells of the gastric mucosa and the epithelial cells of the renal tubules were thought by the authors to be precipitated in degenerated tissue, although they did not explain why no calcification was found in degenerated areas of the thyroid gland, the liver and the skeletal muscles.

Kreitmar and Hintzelmann¹³³ gave 380 mg. of irradiated ergosterol to a cat in 19 days, during which a weight loss of 1.7 Kg. and terminal coma developed before the animal was put to death. Autopsy disclosed calcium deposits in muscle fibers and the stroma of the heart, in the intima and the media of the aorta, in all coats of the stomach, and in the basement membranes of glomeruli and convoluted tubules, the cells and the lumens of straight tubules, and the afferent glomerular arterioles of the kidneys.

Kreitmar and Moll,¹³⁴ by giving a proprietary preparation of irradiated ergosterol in oil to mice, rats, guinea pigs, rabbits, cats, and dogs, were able to produce calcification of the myocardium, the systemic arteries, the lungs, the stomach, the adrenal glands, the kidneys and the intercostal muscles as well as ulcers of the small intestine and severe atrophy of the spleen.

Brand and Holtz¹³⁵ gave 20 mg. of irradiated ergosterol in sesame oil daily to 96 rats divided into four groups, three of which were killed at five, ten and thirty-eight days. The average serum calcium value (11.8 to 16.9 mg. per hundred cubic centimeters) and phosphorus value (9.8 to 13.1 mg. per hundred cubic centimeters) rose with increasing doses, compared with a value of 9.8 mg. for calcium and a value of 8.3 mg. for phosphorus obtained on a control group of 24 rats divided in half and killed at ten and thirty-nine days. The fourth experimental group received 20 mg. of the drug daily for nine days, then only sesame oil for the next sixteen days, and were killed at thirty-eight days. The average serum calcium and phosphorus values for this group were

132. Cantarow, A.; Stewart, H. L., and Housel, E. L.: *Endocrinology* **22**:13, 1938.

133. Kreitmar, H., and Hintzelmann, U.: *Arch. f. exper. Path. u. Pharmacol.* **137**:203, 1928.

134. Kreitmar, H., and Moll, T.: *München. med. Wchnschr.* **75**:637, 1928.

135. Brand, T., and Holtz, F.: *Ztschr. f. physiol. Chem.* **185**:217, 1929.

12.4 mg. and 9.5 mg per hundred cubic centimeters. In the experimental animals calcification was found in the cardiac muscle fibers, in the media of the coronary arteries, in the elastic fibrils of the alveolar walls of the lungs, in the gland crypts and the stroma of the gastric mucosa, in the medulla of the adrenal glands and in the glomerular capsules and the basement membranes, epithelial cells and lumens of the tubules of the kidneys.

Two dogs given 5 to 7 million antirachitic units of a proprietary preparation of irradiated ergosterol in oil in eight weeks by Demole and Fromherz ¹³⁶ showed a 20 per cent loss of body weight, and at autopsy calcium deposits were found in the endocardium of the left auricle and ventricle, in the intima of the pulmonic veins, in the intima and the media of the arch of the aorta, and in the glomeruli and the tubules of the kidneys.

Herzenberg ¹³⁷ gave a proprietary preparation of irradiated ergosterol in oil in doses of 6 to 10 mg. to 12 adult rats for sixteen to forty-six days. The animals lost 20 to 25 per cent of their body weight, and constitutional symptoms developed. Necropsy revealed heavy calcium deposits in relation to necrotic areas in the heart muscle, the media of the aorta, the muscle of blood vessels, the muscle of the stomach and the diaphragm. Calcium deposits were noted in the elastic tissue of blood vessels, in the bronchial mucosa and the elastic tissue of the septums of the lungs, in the lamina propria of the trachea and in the glomerular capsules and basement membranes of the tubules of the kidneys. She was unable to decide whether necrosis of smooth muscle or injury with calcification of elastic fibrils was primary.

Smith and Elvove ¹³⁸ gave 27 full-grown rabbits, orally or intramuscularly, irradiated ergosterol in doses of 1 to 10 mg. daily or total amounts of 29 to 310 mg. With doses over 2 mg. daily, there was a high mortality, and abundant calcium deposits were seen in the media of the thoracic aorta, in the bronchial cartilages and the interalveolar septums of the lungs and in the convoluted tubules and as casts in the straight tubules of the kidneys. Also seen was diffuse interstitial nephritis accompanying the calcium deposits. Chemical analysis of the lungs and the kidneys revealed an enormous increase of calcium, especially in the latter. The salt deposited was calcium phosphate. A high inorganic phosphorus level was produced by the larger doses of irradiated ergosterol. Elevated serum concentrations of phosphorus and calcium, even though the latter was not high in absolute amount, resulted in much tissue calcification. When the serum concentration of phosphorus was normal or low, abnormal deposits of calcium were not found in the

136. Demole, V., and Fromherz, K.: *Arch. f. exper. Path. u. Pharmakol.* **146**:347, 1929.

137. Herzenberg, H.: *Beitr. z. path. Anat. u. z. allg. Path.* **82**:27, 1929.

138. Smith, M. I., and Elvove, E.: *Pub. Health Rep.* **44**:1245, 1929.

tissues no matter how high the serum calcium was. The authors concluded that hypercalcaemia alone was not enough to account for abnormal tissue deposits of calcium and that coincident high inorganic serum phosphorus was essential to such calcification.

Shohl, Goldblatt and Brown¹³⁹ fed 4 mg. of a proprietary preparation of irradiated ergosterol in oil daily for five to seven days to 10 rats, beginning when these were 7 weeks old. Autopsy showed: calcification in the myocardium, the cardiac blood vessels, the mucosa and muscle of the stomach and the tubules and blood vessels of the kidneys; parenchymatous degeneration in the liver and the kidneys; necrosis in the myocardium and in renal tubules, and infiltration-proliferation in the heart and the gastric smooth muscle. Two rats on a phosphorus-deficient diet and 2 on a calcium-deficient diet, as well as 5 control rats, showed no calcification. The authors thought that the degenerative changes preceded calcification in the heart, the stomach and the kidneys, although they conceded that true metastatic calcification was responsible for some of the deposition of calcium in these organs. They did not explain why calcium was not deposited in areas of the liver affected by parenchymatous degeneration.

Gough, Duguid and Davies¹⁴⁰ studied two groups of 18 full-grown young rats each, one on an acid diet, the other on an alkaline diet, both diets being equivalent in phosphorus content but low in calcium content. Twelve rats in each group also received daily 20,000 units of oral vitamin D as calciferol. Urinary calcium was relatively higher with the alkaline diet but was increased with both diets on the addition of calciferol. Renal calcium, determined on the left kidney, was highest in the rats on the alkaline diet-calciferol combination. The right kidney showed a gross white or yellow granular corticomedullary junction. Microscopically, calcification was most abundant in the animals getting calciferol and more severe with the alkaline diet. The chemical and histologic calcific changes were parallel. Nephrosis affected the rats on an acid diet and was enhanced by calciferol. The nephrotic changes included shrunken or necrotic epithelial cells, replacement fibrosis of atrophic tubules and dilatation of some remaining tubules and were thought by the authors to be independent of the calcific changes. Two other groups of 12 rats each were studied, one fed an acid diet, the other fed an alkaline diet. Six rats in each group also received calciferol. The average excretion of urinary phosphorus was relatively elevated with the acid diet, lowered with the alkaline diet and much lowered with both diets on the addition of calciferol. The acid diet-calciferol combination resulted in the largest content of renal phosphorus.

139. Shohl, A. T.; Goldblatt, H., and Brown, H. B.: *J. Clin. Investigation* 8:505, 1930.

140. Gough, J.; Duguid, J. D., and Davies, D. R.: *Brit. J. Exper. Path.* 14:137, 1933.

Tanaka ¹⁴¹ injected a calcium lactate solution intraperitoneally into a rabbit and observed deposition of calcium in the peritoneum, in the muscle fibers of the heart, in the intima and the media of the aorta, in the lumens of the straight tubules of the kidneys and in the skeletal muscles of the extremities. He also gave 4 dogs intravenously calcium lactate and sodium phosphate seven to eighteen days after removing 200 cc. of blood by vein from each. Within twenty-four hours after the injection, autopsy revealed calcification of epithelial cells in the renal tubules in 4, of muscle fibers in the heart in 4, of the capsule of the spleen or the liver in 2 each, of the endocardium in 1 and of the stomach in 1. Hemorrhages involved mucous and serous surfaces in all 4.

Rabl ¹⁴² fed 17 mice for three to five days on an alternating acid and alkaline diet containing one part of tertiary calcium phosphate. Calcification involved: heart muscle fibers; all coats of the aorta and large arteries; elastic fibrils, bronchi, bronchial cartilages and veins in the lungs; the tunica propria, the gland cells and lumens of glands in the stomach; the basement membranes and the lumens of the renal tubules.

Butler ⁸⁹ gave 9 mice an acid diet for five to sixteen days and found calcium deposits in the lungs, the stomach and the kidneys varying from none or a trace to grades III or IV. Six other mice fed an alkaline diet for eight to fourteen days showed calcification of the same organs varying from none or a trace to grades II or III. Eleven mice fed an alternating acid and alkaline diet for four to sixteen days had calcium deposits in these organs varying from none or a trace to grades III or IV. Microscopic study revealed calcification of the alveolar septums, the intima of veins and arteries and the basement membranes of the bronchioles in the lungs, of the mucosal glands in the stomach and of epithelial cells and basement membranes in the renal tubules. In 5 animals deposition of calcium was found in the myocardium. In 17 control mice no calcification was observed except in a small area in the renal collecting tubules. No inflammatory reaction accompanied the calcium deposits in the experimental animals.

Dreyfuss ¹⁴³ gave tertiary calcium phosphate to four groups of mice. Of 6 mice fed an alternating acid and alkaline diet for six to fifty-four days, calcification affected the ventricular cardiac muscle fibers in 5, the bronchial cartilages in 1, the gastric mucosa in 6 and lumens of the renal tubules in 6. Of 7 mice fed an acid diet for two to forty-five days, calcium deposits involved the cardiac muscle in 6, the intima and the media of the blood vessels and the bronchial cartilages of the lungs in 7, the gastric mucosa in 6 and the lumens of the renal tubules in 7. The cardiac and renal deposits were much heavier than in the group fed the alternating diet. Of 6 mice fed an alkaline diet for eleven to thirty-eight

141. Tanaka, M.: *Biochem. Ztschr.* 35:113, 1911.

142. Rabl, C. R. H.: *Virchows Arch. f. path. Anat.* 245:542, 1923.

143. Dreyfuss, W.: *Beitr. z. path. Anat. u. z. allg. Path.* 76:254, 1926-1927.

days, calcium deposits marked the cardiac muscle fibers in 2, the intima of blood vessels and the bronchial cartilages of the lungs in 3, the muscularis of the stomach in 1 and the kidneys in 5. The heart, the lungs and the stomach were much less affected than in the groups on the alternating or the acid diets and the kidneys were less involved than in the group on the alternating diet. Of 5 mice fed a neutral diet for an unstated period, calcification involved a few cardiac muscle fibers in 1, a large vein and a bronchial cartilage of the lungs in 1 and the kidneys in 5. The stomach was not calcified in any of the 5 mice in this group, in which renal calcification was about as intense as that in the animals fed the alkaline diet.

Kleinmann¹⁴⁴ fed 57 adult mice various diets. The most convincing results were observed in three groups. Of 4 mice fed an alternating acid and alkaline diet for eleven to nineteen days, calcium deposits were noted in cardiac muscle fibers in 1, in the walls of large cardiac blood vessels in 3, in the elastic fibrils of the alveolar walls of the lungs in 4, in the gastric mucosa in 2, and in the lumens of the renal tubules in 2. Of 4 mice fed an acid diet for sixteen to thirty-two days, calcification involved cardiac muscle fibers in 4, the alveolar walls of the lungs in 4, the gastric mucosa in 1, and the lumens and basement membranes of the tubules and the stroma of the kidneys in 4. Of 2 mice fed an alternating acid and alkaline diet (supplemented with calcium chloride instead of with calcium phosphate as in the other two groups) for five and twelve days, calcium deposits affected cardiac muscle fibers and stroma in 2, the alveolar walls of the lungs in 2, the gastric mucosa in 1 and the epithelial cells and the lumens of the tubules and the stroma of the kidneys in 2.

Stephens and Barr¹⁴⁵ fed adult rats various diets and put all animals to death after fifteen days. Of 4 rats on an acid, high calcium, high phosphorus diet, the heart was calcified in 2, the pulmonary, renal and gastric arteries in 3, the stomach in 2 and the kidneys in 4. Of 4 rats fed an alternating acid and alkaline, high calcium, high phosphorus diet, the pulmonary arteries were calcified in 3 and the kidneys in 4. The heart and stomach were not affected by calcification. Of 5 rats fed an alkaline, high calcium, high phosphorus diet, 5 fed a neutral, high calcium, high phosphorus diet, 12 fed an acid, high calcium diet, 6 fed an alternating acid and alkaline, high calcium diet, 6 fed an acid, high phosphorus diet and 6 control rats calcification was absent from the viscera in all. Microscopically, the calcific deposits were chiefly extra-cellular, were usually encircled by lymphocytes and consisted mainly of tertiary calcium phosphate as shown by chemical tests. The deposits were found in the myocardium, in the intima and the media of the pulmonary, renal and gastric arteries, in the muscle coat of the stomach and

144. Kleinmann, H.: *Virchows Arch. f. path. Anat.* **268**:686, 1928.

145. Stephens, D. J., and Barr, D. P.: *Proc. Soc. Exper. Biol. & Med.* **30**:920, 1933.

in the tubules and the interstitial tissue of the kidneys. Calcium was found in bronchial cartilages in both experimental and control animals. The walls of the alveoli of the lungs were not specifically described as affected by calcification.

Summary.—Metastatic calcification has been produced in dogs,¹⁴⁶ guinea pigs,¹²⁹ and rats¹⁴⁷ by injections of parathyroid extract. The organs involved by deposition of calcium were usually those affected in human beings with primary parathyroid neoplasm, although chronic experiments comparable to the spontaneous disease in man were not observed. Degenerated liver cells were found,¹⁴⁶ but calcification was observed in only one experiment.¹²⁸ The degenerative, inflammatory and fibrotic changes accompanying the calcification in the kidneys were emphasized.¹³¹ Elevated serum calcium levels were noted,¹⁴⁸ but attention to inorganic serum phosphorus was lacking.

Large doses of vitamin D have caused metastatic calcification in rats,¹⁴⁹ dogs,¹⁵⁰ cats,¹⁵¹ rabbits,¹⁵² guinea pigs¹³⁴ and mice.¹³⁴ The deposits were ordinarily distributed as are those observed in human beings affected by metastatic calcification due to vitamin D or other causes, and resembled those closely. Calcium deposits were associated with degenerative changes in two experiments.¹⁵³ Degenerative and fibrotic changes were severe in the kidneys of rats given an acid diet and vitamin D.¹⁴⁰ Diffuse interstitial nephritis accompanied the renal calcification.¹³⁸ Calcium phosphate¹³⁸ was the salt deposited in the lungs and the kidneys, the latter organs containing more of the compound. The calcium and the inorganic phosphorus of the serum were increased in 2 instances,¹⁵⁴ and were increased further as more vitamin D was given. The amounts of urinary and renal calcium paralleled each other and were highest when an alkaline diet was given with vitamin D.¹⁴⁰ The urinary phosphorus was lowered to the greatest degree by an alkaline diet plus vitamin D, and the renal level of phosphorus with the same combination was lower than the maximal level of renal phosphorus reached when an acid diet and vitamin D were employed.

Minerals have been used to produce metastatic calcification in the mouse,¹⁵⁵ the rabbit,¹⁴¹ the dog¹⁴¹ and the rat.¹⁴⁵ In distribution and character the calcific deposits were similar to those seen in animals

146. Hueper.¹²⁷ Learner.¹²⁸ Cantarow.¹³²

147. Chown, Lee and Teal (footnotes 130 and 131).

148. Learner.¹²⁸ Cantarow and others.¹³²

149. Kreitmar and Moll.¹³⁴ Brand and Holtz.¹³⁵ Herzenberg.¹³⁷ Shohl and others.¹³⁹ Gough and others.¹⁴⁰

150. Kreitmar and Moll.¹³⁴ Demole and Fromherz.¹³⁶

151. Kreitmar and Hintzelmann.¹³³ Kreitmar and Moll.¹³⁴

152. Kreitmar and Moll.¹³⁴ Smith and Elvove.¹²⁸

153. Herzenberg.¹³⁷ Shohl and others.¹³⁹

154. Brand and Holtz.¹³⁵ Smith and Elvove.¹³⁸

155. Butler.³⁹ Rabl.¹⁴² Dreyfuss.¹⁴³ Kleinmann.¹⁴⁴

receiving parathyroid extract or vitamin D, except that degenerative tissue changes were not observed. Tertiary calcium phosphate was demonstrated in the calcified tissues by chemical test.¹⁴⁵ Except for one experiment of a rather acute nature,¹⁴¹ the work with minerals has been based on a dietary approach.¹⁵⁶ An acid diet, closely followed by an alternating acid and alkaline diet, has been more effective in causing calcification of tissues than an alkaline or a neutral diet. Adequate amounts of calcium and phosphorus, especially of the latter,¹⁴⁵ have been necessary in the diets to produce the calcific changes.

MECHANISMS CONCERNED IN METASTATIC CALCIFICATION

In regard to bone disease as a cause of metastatic calcification, still valid is the original concept of Virchow¹ that the calcium salts derived from the breakdown of osseous tissue enter the blood stream in high concentration, principally as phosphates and carbonates, and are then precipitated in those tissues most susceptible to calcification. Several factors are involved in this process. The first is the composition of bone. According to Taylor and Sheard,¹⁵⁷ the solid phase of bone is composed of minerals of the apatite series of the formula $3\text{Ca}_3(\text{PO}_4)_2 \cdot \text{CaX}_2$, in which X_2 ordinarily represents CO_3 , F_2 , $(\text{OH})_2$, O , or SO_4 and the Ca may be to some extent replaced by Mg. They found that the residual Ca:P ratio of bone is 1.94 and that the roentgenographic patterns and optical properties of bones and naturally occurring minerals of similar composition, including podolite, dahllite and fluorapatite, are the same. In normal rat bone Kramer and Shear¹⁵⁸ found a residual Ca:P ratio of 1.99. They observed that the proportion of carbonate calcium to total calcium was 8 to 10 per cent in the bones of young rats, compared with 15 to 16 per cent in those of adult rats. In primary calcification of the older bones of both young and adult animals, the proportion of carbonate calcium was less and a residual Ca:P ratio of 2.23 was observed, indicating to them¹⁵⁸ that a basic calcium salt is present when bone salts are freshly deposited. Normal adult bone has been found to consist¹⁵⁹ of about 80 per cent calcium phosphate, 13 per cent calcium carbonate, 2 per cent magnesium phosphate and a residue of calcium or magnesium fluoride, oxide, hydroxide or sulfate and magnesium carbonate.

The second factor is the manner in which calcium salts are released from bone. In the destruction of bone by osseous lesions, not only is the organic matrix of the bone directly destroyed to release calcium salts, but hyperemia is one component of the accompanying inflammatory reaction. Blair¹⁶⁰ presented the theory that alternating ischemia and hyper-

156. Rabl,¹⁴² Dreyfuss,¹⁴³ Kleinmann,¹⁴⁴ Stephens and Barr.¹⁴⁵

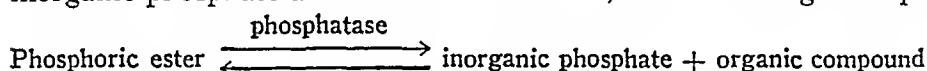
157. Taylor, N. W., and Sheard, C.: *J. Biol. Chem.* **81**:479, 1929.

158. Kramer, B., and Shear, M. J.: *J. Biol. Chem.* **79**:147, 1928.

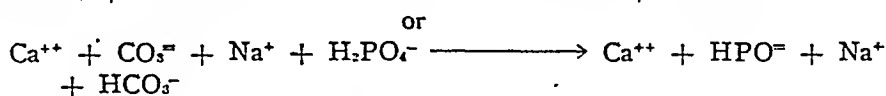
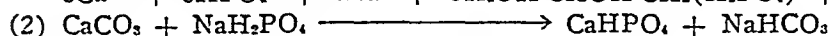
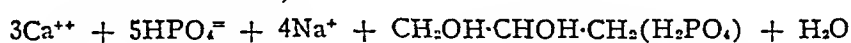
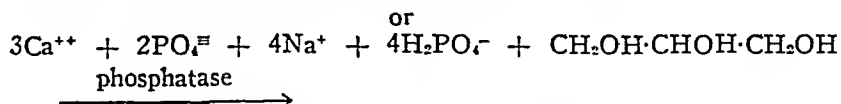
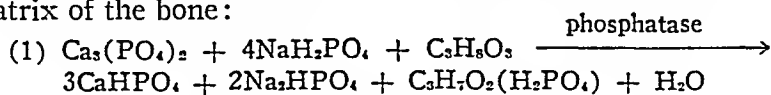
159. Schmidt, C. L. A., and Greenberg, D. M.: *Physiol. Rev.* **15**:297, 1935.

160. Blair, H. C.: *Surg., Gynec. & Obst.* **67**:413, 1938.

emia maintain normal calcification of bone, aid in the healing of fractures and are produced by contraction and relaxation of skeletal muscles. Grieg¹⁶¹ stated that if the circulation is maintained within normal limits, bone remains unchanged, but that definite hyperemia causes bone to undergo rarefaction, decalcification and osteoporosis, contrasted with ischemia, which results in calcification and ossification of bone. As bone is destroyed, the organic matrix may furnish protein molecules with which calcium ions may combine, although increased concentration of hydrogen ions could inhibit this process to some extent by driving it in the direction of ionization of calcium proteinate so formed. With the increasing acidity of the inflammatory process accompanying destruction of bone, the high local concentration of carbon dioxide could enhance the solution¹⁶⁰ of calcium salts. Kay¹⁶² demonstrated a rise in the plasma phosphatase in cases of osteomyelitis, osteitis deformans, metastatic carcinoma of bone, sarcoma of bone, osteitis fibrosa cystica and other conditions. He expressed the opinion that the increase of plasma phosphatase is probably due to the bone disease, possibly because it is produced in excessive amounts in an attempt to compensate for the bony lesion. Kay¹⁶³ concluded that the phosphatase derived from bone is able to hydrolyze many monophosphoric esters and that such esters, which form soluble calcium salts, may serve as a substrate for the precipitation of calcium phosphate in ossification. On the other hand, phosphatase may be able to synthesize¹⁶³ phosphoric esters from organic compounds, e. g., glycerol, under conditions in which even low concentrations of inorganic phosphate are found in the tissues, thus reversing the equation



By this mechanism, calcium salts could be transferred from bones with directly destructive lesions according to the following equations, which employ glycerol as a sample organic compound, the sodium phosphate buffers of the blood, and the calcium orthophosphate ($\text{Ca}_3[\text{PO}_4]_2$) and calcium carbonate (CaCO_3) released by the breakdown of the organic matrix of the bone:



161. Grieg, D. M., cited by Blair.¹⁶⁰

162. Kay, H. D.: J. Biol. Chem. 89:249, 1930.

163. Kay, H. D.: Physiol. Rev. 12:384, 1932.

The release of magnesium ions by the breakdown of the osseous tissue would catalyze¹⁶⁴ the action of the phosphatase.

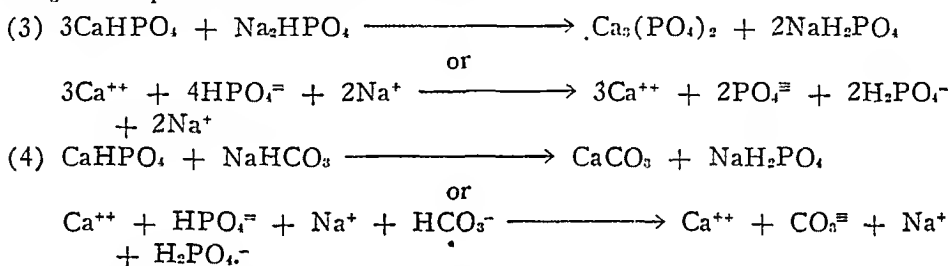
The third factor involved is the blood transport of calcium, phosphorus and magnesium whereby these elements reach the tissues susceptible of being calcified in the metastatic process. Tertiary calcium phosphate and calcium carbonate from the bones may be transformed as indicated into monohydric calcium orthophosphate (CaHPO_4), which is transported in the blood as suggested by the experiments of Shear and Kramer.¹⁶⁵ On mixing solutions containing calcium with solutions containing phosphate so that the resulting solution was acid, they observed the formation of a precipitate, CaHPO_4 . Inorganic serum solutions were adjusted to a p_{H} of 7.3 with calcium to the concentration of 10 mg. per hundred cubic centimeters and phosphorus to concentrations varying from 1.5 to 6.0 mg. per hundred cubic centimeters. When the $\text{Ca} \times \text{P}$ product was 40, calcification occurred, but when this figure dropped to 30, no calcification was obtained. The critical value was 35. When the solubility product constant ($K_{\text{s.p.}}$) of $(\text{Ca}^{++}) \cdot (\text{HPO}_4^{=})$ was between 2.7×10^{-6} and 4.0×10^{-6} , the empiric product of $\text{Ca} \times \text{P}$ was 40 to 60. This constant was lowered in proportion to the drop of the $\text{Ca} \times \text{P}$ product. When the p_{H} of the serum differed to any degree from 7.3, the CaHPO_4 was not held in solution. When the p_{H} dropped to 7 or slightly below, the solubility product constant was so low that the ion products of CaHPO_4 were undersaturated and calcification was not obtained even with a $\text{Ca} \times \text{P}$ product as high as 50. They concluded that normal serum is probably very near to saturation with CaHPO_4 . By shaking crystalline CaHPO_4 with inorganic serum solutions, Shear, Washburn and Kramer¹⁶⁶ found the $(\text{Ca}^{++}) \cdot (\text{HPO}_4^{=})$ ion product at equilibrium, even though the solid phase was in excess and was independent of the initial concentration of Ca and P. The mean value for the solubility product constant of CaHPO_4 at 38 C. was 3.4×10^{-6} . They also observed that inorganic serum solutions with an empiric $\text{Ca} \times \text{P}$ product of less than 50 were undersaturated with respect to CaHPO_4 , while those with a value above 50 were supersaturated, making 50 the critical level at which calcification occurred. In addition to CaHPO_4 , serum contains calcium proteinate and ionic calcium; the calcium combined with protein is combined mainly with the albumin fraction of the serum.¹⁵⁹ Phosphorus is present in serum as inorganic phosphate, nucleotides, glycerophosphate, phosphoric esters, glycerophosphoric acid and phospholipids.¹⁵⁹ Magnesium exists in serum in the same three states as calcium.¹⁵⁹

164. Jenner, H. D., and Kay, H. D.: *J. Biol. Chem.* **93**:733, 1931.

165. Shear, M. J., and Kramer, B.: *J. Biol. Chem.* **79**:125, 1928.

166. Shear, M. J.; Washburn, M., and Kramer, B.: *J. Biol. Chem.* **83**:697, 1929.

The fourth factor involved is the mechanism by which calcium salts are precipitated in those tissues especially prone to be alkaline because of the low carbon dioxide content or, conversely, because of the high oxygen content of the blood bathing them—such as the capillaries of the alveoli, the venules and the veins of the lungs, the left chambers of the heart, and the systemic arterial tree. In those organs in which acids are excreted, such as the lungs (CO_2), the gastric mucosa (HCl) and the kidneys (acid PO_4), the tissues are rendered alkaline and similar precipitation of calcium salts occurs.³ Since serum is normally saturated¹⁶⁶ with respect to CaHPO_4 and an alkaline p_{H} favors¹⁶⁵ the holding of this compound in solution, the following reactions could express the means by which CaHPO_4 is changed to $\text{Ca}_3(\text{PO}_4)_2$ and CaCO_3 for deposition in the soft tissues:



The monosodium acid phosphate (NaH_2PO_4) could be returned to the bone and would facilitate the reactions in equations (1) and (2) for the transformation of $\text{Ca}_3(\text{PO}_4)_2$ and CaCO_3 of the bone to CaHPO_4 for blood transport. The glycerophosphates or other phosphoric esters in the serum and in the tissues becoming calcified could be an available substrate for the action of phosphatase elevated in the serum¹⁶² toward hydrolysis¹⁶⁷ of these esters and the production of phosphate ions to enhance calcification. However, no evidence has been found to prove that phosphatase acts in tissues undergoing calcification by the metastatic process, even though the low concentration of hydrogen ions in these tissues would favor its action at an optimal p_{H} of 9 as compared with a p_{H} of 5, which inactivates this enzyme.¹⁶³ The renal cortex has a high degree of phosphatase activity,¹⁶⁸ which might be concerned with the deposition of calcium salts in the kidneys by the same mechanism. In experiments on frog muscle and canine carotid arteries, which were injured, Burge and co-workers¹⁶⁹ demonstrated that the injured portion was rendered electronegative to the uninjured portion. Increased phosphate was observed in the injured part of the frog muscle by the use of ammonium molybdate. When calcium chloride or barium chloride in twice-normal solution was applied to the injured parts, precipitation of

167. Robison, R.: *Biochem. J.* **17**:286, 1923.

168. Kay, H. D.: *Biochem. J.* **20**:791, 1926.

169. Burge, W. E.; Orth, O. S.; Neild, H. W.; Ash, J., and Krouse, R.: *Arch. Path.* **20**:690, 1935.

calcium phosphate or of barium phosphate resulted, and the current of a few microamperes was abolished. They concluded that calcification of arteries may result from a combination of positively charged calcium ions of the blood with negatively charged phosphate ions in the tissues, so that calcium phosphate is precipitated. This mechanism could be operative in metastatic calcification of tissues, particularly after direct calcification had already involved them, thus allowing phosphate ions to be released toward further deposition of calcium phosphate.

The fifth factor involved concerns the types of salts deposited in the soft tissues and the ratio of Ca:P in them. Kramer and Shear¹⁷⁰ examined 7 specimens of tissues pathologically calcified by the dystrophic process and found a residual Ca:P ratio of 1.86 to 2.01. In three calcified fibroids, values of 2.18 to 2.23 were noted. Similar quantitative studies on tissues pathologically calcified by the metastatic process have not been found, but the supposition that this ratio would be the same is allowable. Among the patients with bone disease and metastatic calcification, calcium carbonate was found in the lungs of 5, the hearts of 3, the kidneys of 2, the gastric mucosa of 1 and the skin of 1. Calcium phosphate was noted in the lungs of 3, the gastric mucosa of 1, the heart of 1 and the kidneys of 1.

Impaired in chronic renal disease, because of the destruction of renal parenchyma, is the ability of the body to excrete inorganic phosphorus, as well as the phosphate of phosphoric esters through renal phosphatase activity, by way of the kidneys, the main avenue whereby phosphorus compounds are excreted¹⁵⁹ as acid phosphates. Thus phosphate is built up in the blood to levels higher than normal, even to extreme levels.⁶¹ With this rise in phosphate, a reciprocal drop in blood calcium occurs to maintain the $(\text{Ca}^{++}) \cdot (\text{HPO}_4^{=})$ solubility product constant and the empiric $\text{Ca} \times \text{P}$ product. In an effort to enhance the excretion of phosphate in the greatly reduced surviving renal parenchyma, the parathyroid glands become enlarged as observed in 15 of the 23 patients with chronic renal disease and metastatic calcification. Eleven of them showed diffuse chief cell hyperplasia of the parathyroid glands, characteristic of chronic renal disease.⁴⁹ That this same abnormality of the parathyroid glands may result from inadequate absorption of calcium and phosphate from the intestine has been little appreciated.¹⁷¹ Through the overactivity of the hyperplastic parathyroid glands in chronic renal disease, the blood calcium is increased¹⁷² so that the serum becomes supersaturated with both calcium and phosphate and precipitation of calcium salts occurs in the soft tissues. The level of phosphate is more important, for although

170. Kramer, B., and Shear, M. J.: *J. Biol. Chem.* **79**:121, 1928.

171. Mulligan, R. M.: *Arch. Path.* **40**:182, 1945.

172. Hubbard and Wentworth.³⁸ Smyth and Goldman.⁴⁴ Price and Davie.⁴⁵ Brown and Ginsberg.⁵¹ Herbert and others.⁵²

normal,⁵⁹ normal or depressed⁵⁸ or even decreased⁴⁸ serum calcium values may be observed, metastatic calcification occurs if the phosphate in the serum is elevated. The calcium of the blood and the accompanying phosphate and carbonate ions have their source in the bones, in which osteitis fibrosa cystica was the lesion in 7 patients, rickets in 4 and osteoporosis in 2 patients of the 13 with chronic renal disease whose bones were examined histologically. The direct action of parathyroid hormone; the hyperemia attendant on passive visceral hyperemia caused by a hypertrophied failing heart, often seen in chronic renal disease; the high local carbon dioxide content resulting from decreased carbon dioxide-combining power of the blood⁶⁶; the local increase of protein due to the breakdown of organic bony matrix; the elevated phosphatase⁶² all may play etiologic roles when calcium salts are being mobilized from the bones in chronic renal disease. Equations (1) and (2) could explain the removal of these salts as in the case of bone disease. With calcium and phosphate as well as carbonate being increased in the blood as they are withdrawn from the bones, the elevation of tissue p_H , the saturation of the serum with monohydric calcium orthophosphate (CaHPO_4), the action of alkaline phosphatase on phosphoric esters, equations 3 and 4 as given in a foregoing paragraph, and initial injury of tissue by direct calcification would have to be considered in explaining tissue calcification in the case of chronic renal disease as they are in the case of bone disease. Of the 23 patients with chronic renal disease whose cases were reviewed, 43 per cent had calcified kidneys. Although elevated concentration of hydrogen ions of the blood is a frequent feature of chronic renal disease, episodes of vomiting, also observed, may result in enough chloride being lost from the blood to cause temporary elevation of the blood bases, so that tissue alkalinity, especially in those sites prone to be alkaline, is intermittently raised. This would tend to have an effect like that of alternating acid and alkaline diets¹⁷³ in causing calcium to be deposited in the tissues. In the heart Schmidt³⁷ found calcium phosphate and a preponderance of calcium in the left ventricle as compared with the right ventricle. Smyth and Goldman⁴⁴ observed periarticular deposits of calcium phosphate and a ratio of calcium to phosphorus of about 2:1 in the long bones of their patient. Pons and Pappenheimer⁵⁰ noted that the amount of calcium in the kidneys of their second patient was about one hundred and thirty times the amount found in normal kidneys.

In primary hyperparathyroidism¹⁷⁴ resulting from a chief cell or other type of adenoma of a parathyroid gland or from diffuse hyperplasia of wasserhelle cells of two or more parathyroid glands, both calcium and phosphate are withdrawn from the bones,¹⁵⁹ conspicuously from the

173. Butler.³⁰ Rabl.¹⁴² Dreyfuss.¹⁴³ Kleinmann.¹⁴⁴ Stephens and Barr.¹⁴⁵

174. Castleman, B., and Mallory, T. B.: *Am. J. Path.* **11**:1, 1935.

trabeculae,¹⁷⁵ and are discharged into the blood by the great activity of the neoplastic parathyroid tissue. The mechanisms operative in the removal of calcium salts in bone disease and chronic renal disease could be active in primary hyperparathyroidism, such as the hyperemia associated with new-formed osteoid tissue,⁷³ increase of alkaline phosphatase¹⁰² and a high local concentration of protein following the breakdown of original organic bony matrix. Equations (1) and (2) could also apply. By the action of parathyroid hormone the blood calcium is raised¹⁷⁰ and the blood inorganic phosphorus lowered,^{170a} although both are excreted by the kidneys in increased amounts,¹⁷⁰ especially phosphorus. Fecal excretion of calcium is not affected.^{170a} Muscle weakness⁸⁷ is caused when the calcium ions of the blood are increased; renal calculi,¹⁷⁴ when calcium phosphate is being excreted in large amounts by the kidneys; osteitis fibrosa cystica,¹⁷⁴ when bone is being resorbed and replaced by fibrous and osteoid tissue,⁷³ and metastatic calcification, when calcium salts are being deposited in the soft tissues. In patients with primary hyperparathyroidism¹⁷⁴ the formation of renal calculi precedes osteitis fibrosa cystica by a long enough interval to indicate that the calcium salts are withdrawn from the bones at an early stage of the disease and that the osseous lesion is manifest only in the later stages. The lesion in the bones of 17 of the 21 patients with primary neoplasm of the parathyroid glands reviewed was osteitis fibrosa cystica in 14, osteomalacia in 1, osteoporosis in 1 and osteosclerosis in 1. A superb presentation of the genesis of osteitis fibrosa cystica has been given by Dawson and Struthers.⁷³ The experimental production of this lesion has been observed in guinea pigs by Jaffe, Bodansky and Blair¹²⁰ and in albino rats by Selye.¹⁷⁷ The kidneys of 90 per cent of the 21 patients with primary parathyroid neoplasm and metastatic calcification were calcified to various degrees, and more or less inflammatory reaction, degenerative changes and fibrosis accompanied the deposition of calcium salts. Apparently the kidneys are the first site of calcification in primary hyperparathyroidism, and when the destructive alterations accompanying this calcification have supervened, the amount of normal renal parenchyma is greatly reduced and the excretion of phosphate especially is impaired as in primary chronic renal disease. When this occurs, or even when the blood calcium is raised^{170a} above a critical level of 14 to 15 mg. per hundred cubic centimeters of serum, the excretion of urinary phosphate falls and the level of blood phosphate is abnormally high. With the blood calcium already increased, the critical level of both ions is raised in the blood to the point at which precipitation occurs in those

175. Bauer, W.; Aub, J. C., and Albright, F.: *J. Exper. Med.* **49**:145, 1929.

176. (a) Albright, F.; Bauer, W.; Ropes, M., and Aub, J. C.: *J. Clin. Investigation* **7**:139, 1929. (b) Schmidt and Greenberg.¹⁵⁰

177. Selye, H.: *Endocrinology* **16**:547, 1932.

tissues, besides the kidneys, prone to direct calcification. With the reduction of renal parenchyma by calcific and secondary pathologic changes, the already neoplastic parathyroid tissue is incited to further efforts toward facilitating excretion of phosphate and calcium is further built up in the blood by the action of parathyroid hormone. Thus a vicious circle is set up by renal damage superimposed on the original overactivity of the cells of an adenoma or of hyperplastic water-clear cells. Calcification of the soft tissues in primary hyperparathyroidism depends on the hydrogen ion concentration of the tissues calcified, the saturation of serum with monohydric calcium orthophosphate (CaHPO_4), the local action of phosphatase elevated in the serum (and in the surviving renal parenchyma) on phosphoric esters, and probably to some degree on actual primary injury of tissue as described by some authors.¹⁷⁸ Equations 3 and 4 may also be operative. The renal calculi found in primary hyperparathyroidism are usually calcium phosphate.¹⁷⁴ Chemical data on tissue deposits of calcium salts have been absolutely lacking.

Excessive doses of vitamin D have produced elevation of both calcium¹⁷⁹ and inorganic phosphorus¹⁵⁴ in the serum by causing both to be withdrawn from the bones.¹⁵⁹ In dogs given large doses of irradiated ergosterol, Taylor and Weld^{179a} observed a fall in fecal calcium, a rise in serum calcium, an increased urinary excretion of calcium and a negative calcium balance even when a low calcium diet was fed. The net result of excessive doses of vitamin D is retention of calcium¹⁸⁰ and phosphorus,¹⁸¹ so that the solubility product constant of $(\text{Ca}^{++}) \cdot (\text{HPO}_4^{=})$ is relatively easily exceeded and precipitation of both occurs in those soft tissues susceptible to metastatic calcification. Calcium salts are removed from the bones by vitamin D mainly through osteoclasia.¹⁸² Jones and Robson^{182b} observed that in rats 68 to 138 days old which had been given irradiated ergosterol for fifty-two to sixty-three days, the femur, the tibia and the fibula showed extensive osteoclastic resorption, especially of the cortical bone of the diaphyses. The process did not seem to be simple halisteresis but destruction and removal of the entire bone matrix brought about by the action of osteoclasts. The process began along the inner margin of the cortex of the shaft and extended outward. Where bone had been destroyed, the

178. Oliver.⁸⁴ Hueper.¹²⁷ Cantarow and others.¹³²

179. (a) Taylor, N. B., and Weld, C. B.: *Brit. J. Exper. Path.* **13**:109, 1932.
(b) Brand and Holtz.¹³⁵ (c) Smith and Elvove.¹³⁸

180. Brand and Holtz.¹³⁵ Smith and Elvove.¹³⁸ Gough and others.¹⁴⁰ Schmidt and Greenberg.¹⁵⁹ Taylor and Weld.^{179a}

181. Brand and Holtz.¹³⁵ Smith and Elvove.¹³⁸ Gough and others.¹⁴⁰ Schmidt and Greenberg.¹⁵⁹

182. (a) Grauer, R. C.: *Proc. Soc. Exper. Biol. & Med.* **29**:466, 1932.
(b) Jones, J. H., and Robson, G. M.: *Am. J. Physiol.* **103**:338, 1933. (c) Mulligan.¹⁰⁹ (d) Schmidt and Greenberg.¹⁵⁹

spaces left were filled up by marrow. Hyperemia caused by this extension of marrow and high local concentration of protein following the destruction of organic bone matrix could help to remove calcium salts from the bones in hypervitaminosis D. Data, especially with reference to human beings, on alkaline phosphatase in this condition are not available to indicate either a positive or a negative role for the enzyme in osseous breakdown. Baumgartner, King and Page¹⁸³ showed bone phosphatase greatly decreased in rabbits 9 to 12 months old which were given a proprietary preparation of irradiated ergosterol in oil for two to five and one half months. Rabbits given excessive amounts of irradiated ergosterol showed no phosphatase activity in arteries which were the sites of calcification.¹⁶³ On the other hand, Page¹⁸⁴ demonstrated decreased phosphatase activity in the bones of rats given injections of a parathyroid extract as contrasted with the elevated serum phosphatase observed by Kay¹⁶² in patients with osteitis fibrosa cystica. Although it has been concluded¹⁶⁵ that the actions of parathyroid hormone and vitamin D are parallel in many ways, this similarity does not imply identity. The differences in the bony lesions, in the changes in the blood calcium and phosphorus and in the effects on the fecal calcium produced by these substances indicate definite variations in their behavior, to name a few. In this laboratory, atrophy¹⁸⁶ of the parathyroid glands has been produced in dogs with neutral or alkaline diets and large doses of vitamin D. The calcification of the soft tissues produced by vitamin D in human subjects has not yielded enough anatomic data to be too significant, but the calcium deposits observed in the kidneys of all 9 patients whose data have been reviewed are probably important. The mechanisms operative in the calcific process could include all those outlined for bone disease, chronic renal disease and primary neoplasm of the parathyroid glands except increased local acidity of the bones. The fact that tissue calcification caused through the action of vitamin D is enhanced by an alkaline diet,¹⁴⁰ by intravenously injected sodium bicarbonate solution¹⁸⁷ or by a high phosphorus diet¹⁸⁸ should be emphasized.

The experimental use of acid and alkaline diets has been successful¹⁷³ in causing metastatic calcification in animals, especially in the case of acid or of alternating acid and alkaline diets. Mineral dietary factors may be significant in the same process in human beings, since calcium

183. Baumgartner, L.; King, E. J., and Page, I. H.: *Biochem. Ztschr.* **213**:170, 1929.

184. Page, I. H.: *Biochem. Ztschr.* **223**:222, 1930.

185. Taylor, N. B.; Weld, C. B.; Branion, H. D., and Kay, H. D.: *Canad. M. A. J.* **25**:20, 1931.

186. Mulligan, R. M., and Stricker, F. L.: Unpublished data.

187. Hess, A. F.; Benjamin, H. R., and Gross, J.: *J. Biol. Chem.* **94**:1, 1931.

188. Shelling, D. H.: *Proc. Soc. Exper. Biol. & Med.* **28**:298, 1930.

salts are more easily absorbed from the intestine and mobilized from the bones when the diet is acid and are absorbed in decreased amounts from the intestine and fixed in the osseous system when the diet is alkaline. However, there is no record of metastatic calcification due to a mineral diet alone having been observed in man.

Combinations of various etiologic factors may be responsible for metastatic calcification. This has been demonstrated in cases of bone disease with chronic renal disease¹⁸⁹ or with hypervitaminosis D,²⁸ in cases of chronic renal disease with hypervitaminosis D⁶⁹ with⁵³ or without¹⁹⁰ an alkaline diet and in cases of primary hyperparathyroidism with hypervitaminosis D⁹⁸ with⁸⁰ or without⁸⁹ an alkaline diet.

CONCLUSION

In human pathology, bone disease, chronic renal disease, primary parathyroid neoplasm and hypervitaminosis D, alone or in combination, have been incriminated as causing metastatic calcification. In animals, parathyroid extracts, vitamin D and mineral diets have been employed to produce metastatic calcification. Concerned in the production of this calcification are the direct effects of destructive lesions on bone, the retention of phosphate in the blood following damage of the renal parenchyma, the demineralization of the skeleton caused by the parathyroid hormone in secondary and primary hyperparathyroidism, the osteoclasts produced by hypervitaminosis D, the chemical composition of bone, the manner in which calcium salts are released from the bones into the blood, the blood transport of calcium salts whereby they reach the soft tissues and the mechanism whereby calcium salts are deposited in those tissues susceptible to metastatic calcification. In the release of the calcium salts of the bones, hyperemia, increased concentration of protein, altered concentration of hydrogen ions and phosphatase activity must be considered. In the mechanism by which calcium salts are deposited in the soft tissues, supersaturation of the serum with calcium and phosphate ions, reduction of the tissue concentration of hydrogen ions, phosphatase activity, and tissue injury due to the initial calcific changes are factors to be borne in mind. Equation reactions have been suggested by which the demineralization of bones and the calcification of the soft tissues may occur.

189. Virchow.¹ Askanazy.² Küttner.⁵ Czech.⁸ Plaue.⁸ Heller.⁸ Kockel.⁹ Bender.¹¹ Scheele and Herxheimer.¹³ Jadassohn.¹⁷ Pari.¹⁸ Barr.²⁴

190. Lightwood.⁴¹ Pollack and Siegal.⁴⁷ Brown and Ginsberg.⁵¹

Obituaries

HAROLD E. ROBERTSON, M.D.

1878—1946

Harold E. Robertson was born Oct. 8, 1878 at Waseca, Minn. He received the degree of Bachelor of Arts in 1899 from Carleton College, Northfield, Minn., and later attended the University of Pennsylvania, from which he obtained the degree of Doctor of Medicine in 1905. After one year as instructor in pathology at Albany Medical College, he felt the need of more training and became assistant pathologist at the Boston City Hospital under the late Dr. F. B. Mallory, for whom he had great respect and lasting admiration. During this period he was an instructor in pathology at Harvard Medical School. In 1907 he returned to his native Minnesota as instructor in pathology and bacteriology at the University of Minnesota, where he became head of the department and full professor of pathology and bacteriology in 1914, which position he retained until 1921. In 1914 and 1915 he studied in Germany under Dr. Ludwig Pick and, following this, under the late Dr. Ludwig Asthoff, with whom he worked on the problem of tetanus. From 1917 to 1919 he was a major in the Medical Corps of the American Expeditionary Force, being stationed at Army Laboratory I for six months, and later at Dijon and at Paris, France. On July 1, 1921 he went to the Mayo Clinic as head of the section on pathologic anatomy, at which time he was also appointed professor of pathology in the Mayo Foundation, Graduate School, University of Minnesota. The latter position he retained at the time of his death.

Dr. Robertson had wide interest in the field of pathologic anatomy and wrote on many subjects, but he was especially interested in pulmonary tuberculosis, ulcers of the duodenum, diseases of the gallbladder and diseases of the gastric mucosa. In 1944 was published his book entitled "Hydronephrosis and Pyelitis (Pyelonephritis) of Pregnancy, Etiology and Pathogenesis, an Historical Review."

As a result of early training under the late Dr. F. B. Mallory, Dr. Robertson retained his interest in histologic technical methods. He was, in his earlier years in Rochester, much interested in the teaching museum and in museum technics, some of which he developed himself. This required much time and, as he felt he was not giving sufficient time to teaching, he helped form a separate department, the medical museum. The weekly clinicopathologic meeting which Dr. Robertson developed

in Rochester was for twenty-three years the outstanding meeting of the week and has been the model for many other conferences throughout the country.

In spite of all these activities, to those of us who remain to carry on, his greatest contribution to medicine and to the Mayo Clinic was his outstanding ability as a teacher. He was a naturally forceful speaker,



H. E. Robertson

HAROLD E. ROBERTSON, M.D.
1878—1946

and to all who listened to him he imparted his own enthusiasm. His strong personality, combined with wide and exact knowledge, gained for him a position of preeminence among his colleagues in the field of pathology. He died in Rochester, Minn., on March 8, 1946 at the age of 67 years.

Notes and News

Appointments, Etc.—In the University of North Carolina School of Medicine, Chapel Hill, K. M. Brinkhaus has been appointed professor and head of the department of pathology; F. L. Rights, assistant professor of bacteriology, and J. B. Graham, instructor in pathology.

N. Goormaghtigh, professor of pathology, University of Ghent, Belgium, will spend next April and May in the United States, under the auspices of the Belgian-American Educational Foundation.

New Cancer Journal.—The British Empire Cancer Campaign announces the publication of the *British Journal of Cancer* as its official organ. The annual subscription is \$8.40; the publisher is H. K. Lewis & Co., 136 Gower Street, London, W. C. 1.

Death.—George T. Caldwell, professor of pathology, Southwestern Medical College, Dallas, Texas, died of coronary occlusion Jan. 20, 1947, aged 64. He



GEORGE T. CALDWELL
1882-1947

received his M.A. degree in chemistry at Ohio State University in 1913. In 1919 he graduated in medicine at Rush Medical College, and in the same year he obtained the Ph.D. degree in pathology at the University of Chicago, where he came under the influence of H. Gideon Wells, whose methods of teaching he followed closely. From the fall of 1919 to 1943 he was professor and head of the department of pathology in Baylor University School of Medicine, Dallas, and since 1943 he occupied the same position in the Southwestern Medical College, in the same city. His wife, the former Janet Anderson, M.D., Baylor University, 1921, and pathologist in Dallas, survives him, as does the daughter, who also is a physician. Dr. Caldwell was an effective and successful teacher, administrator, organizer of hospital laboratories, tissue diagnostician, consultant and, altogether, a highly important factor in the progress of medical education and practice in his part of the country.

Society News.—The College of American Pathologists was organized in Chicago, Dec. 12 and 13, 1946. Frank W. Hartman, Detroit, is the president. The main purpose of the college is "to elevate the standards of the practice of pathology." The college is an outgrowth of the American Society of Clinical Pathologists.

The American Association for Cancer Research will hold its thirty-eighth annual meeting in the Hotel Stevens, Chicago, May 16 and 17 next.

The Society of American Bacteriologists will meet in Philadelphia May 12 to 16 next, with headquarters at the Bellevue-Stratford Hotel.

Army Medical Library Microfilm Service.—This service is now generally available for civilian physicians, institutions and research workers on a cost basis. This means direct access to the library's enormous resources of medical literature. A fee of 50 cents is charged for filming any periodical article in a single volume, regardless of length. Microfilming from monographs is furnished at 50 cents per 50 pages or fraction thereof. Photostats are also available at a charge of 50 cents per 10 pages or fraction thereof. Material filmed may not be reproduced without the permission of the copyright owner. For convenience and to keep bookkeeping costs down, a coupon system has been established. Users may buy any quantity of photoduplication coupons at 50 cents each. Order blanks are available on request. Checks should be made payable to the Treasurer of the United States, and sent to the Army Medical Library, Seventh Street and Independence Avenue, S. W., Washington 25, D. C.

Books Received

CARBOHYDRATE METABOLISM: CORRELATION OF PHYSIOLOGICAL, BIOCHEMICAL AND CLINICAL ASPECTS. By Samuel Soskin, M.D., director of the Michael Reese Hospital Research Institute; medical director, Michael Reese Hospital, Chicago, and professorial lecturer in physiology, University of Chicago; and Rachmiel Levine, M.D., director of metabolic and endocrine research, Michael Reese Hospital, Chicago. Pp. 315, with 75 illustrations. Price \$6. Chicago: University of Chicago Press, 1946.

The authors, who are well known for their researches on carbohydrate metabolism, present in this book an excellent review of the state of knowledge and understanding in this field. The text consists of five parts, with topics as follows: the biochemistry and energetics of carbohydrate metabolism; introductory physiologic considerations; a critical survey of the classic criteria of diabetes; the role of the endocrine glands in carbohydrate metabolism; the integration of physiologic and clinical aspects. At the end of each chapter is a comprehensive bibliography. The book is clearly written and well illustrated. The chapter on insulin answers well the question so often asked of the physician about the mode of action of this substance. The roles played in carbohydrate metabolism by the adrenal cortex, the thyroid gland and the anterior lobe of the pituitary gland are also fully explained. The book will be of value to all who are interested in the scientific as well as practical aspects of carbohydrate metabolism.

TUBERCULOSIS IN THE UNITED STATES. GRAPHIC PRESENTATION. VOLUME 4: MORTALITY STATISTICS FOR URBAN PLACES AND RURAL AREAS IN EACH COUNTY. 1939-1941. Prepared by the staff of the Field Studies Section of the Tuberculosis Control Section of the Tuberculosis Control Division, United States Public Health Service, under the direction of Carroll E. Palmer, M.D. Pp. 190. New York: National Tuberculosis Association, 1946.

THE FIRST HUNDRED YEARS OF THE SMITHSONIAN INSTITUTION, 1846-1946. By Webster P. True, chief of the editorial division of the Smithsonian Institution. Pp. 64, with 41 illustrations. Washington, D. C.: Smithsonian Institution, 1946.

CARNEGIE CORPORATION OF NEW YORK. REPORTS OF OFFICERS FOR THE FISCAL YEAR ENDED SEPT. 30, 1946. Pp. 90. New York: Carnegie Corporation of New York, 1946.

FIVE TYPES OF SO-CALLED BRONCHIAL ADENOMA

A Histopathologic Study

CAPTAIN MACHTELD E. SANO

AND

LIEUTENANT COLONEL RICHARD MEADE Jr.

MEDICAL CORPS, ARMY OF THE UNITED STATES

FIVE CASES have been chosen to illustrate the variation of the histopathologic structure and of the cellular characteristics of the so-called bronchial adenoma. Much has been written in recent years of the cellular origin of bronchial adenoma, of its relationship to embryologic rests¹ and of its behavior.² Even today the controversy as to its potential malignancy is still a point of debate.³ A brief review of the anatomy of the region in which the tumor occurs, i. e., the larger bronchi, will clarify the discussion.

The larger bronchi are lined by several rows of cells: basal cells, intermediate cells, cuboidal cells and ciliated cells. Underlying these layers is a musculoelastic stroma. The crescent cartilages of the bronchi differ in size and shape. They have been described in detail by Miller.⁴ In the bronchi the membranous interval separating the cartilages from each other is mesial. Glandular structures can be found in these intervals. These glands form part of a cluster lying either behind or in front of the bronchial cartilages. Examination of many normal bronchi, as well as of bronchi in cases of diseases of the lungs, shows a similar distribution. The cells lining the mucous glands are relatively tall and triangular, with the rounded tip of the triangle directed toward the lumen. The cytoplasm of these cells is either finely granular or vacuolated and takes a pale basophilic stain. The serous cells have a darker, sometimes finely granular cytoplasm. The component cells of these glands are either mucoid or serous, with the mucoid variety usually predominating

From the Chest Service and the Department of Pathology, Kennedy General Hospital, Memphis, Tenn.

1. Graham, E. A., and Womach, N. A.: *J. Thoracic Surg.* **14**:106, 1945.

2. Clerf, L. H., and Bucher, J. C.: *Ann. Otol., Rhin. & Laryng.* **51**:836, 1942.

3. Jackson, C. L.; Konzelmann, F. W., and Norris, C. M.: *J. Thoracic Surg.* **14**:98, 1945.

4. Miller, W. S.: *The Lung*, Springfield, Ill., Charles C Thomas, Publisher, 1943.

by far. However, occasionally the cell types are more evenly distributed and one may see dark-staining crescents at the terminal portion of the gland. These are sometimes referred to as the demilunes of Giannuzzi.⁵ These serous crescents may not be present in the glands of some lungs. The nuclei of these cells are centrally placed, while in the mucous cell the nucleus is at the base. In the isthmus of the gland the basal cell layer can be clearly visualized. These basal cells have small round nuclei rather than the oval type which one usually finds in this type of gland in other parts of the body. The interstitial stroma is delicate, sometimes hard to identify. In the distal portions of the larger bronchi the glandular structures become progressively smaller and less numerous until in the terminal portions and in the bronchioles they are entirely absent.

The cells which one sees in the bronchial adenoma resemble the different component cells of the bronchial glands and are found in the same anatomic locations. The predominating tumor cell type may vary, however, and this is true in each of the cases to be reported. While in certain parts of the tumor a tendency toward acinous arrangement and the cell relationship may be preserved, in other parts this may be completely lost and the tumor be identical with an undifferentiated cell carcinoma. A detailed description of the cellular structure of the tumor is made in the report of each case. In the normal bronchial glands the mucicarmine stain was not always typical. It was negative in all the tumor sections, so that no differentiation into cylindromas was made.⁶

Local removal of bronchial adenoma is not advocated (Graham and Womach⁷), though a few are still in favor of this mode of treatment (Jackson and co-workers³). As the first case and figure 1 illustrate, the tumor, pathologically speaking, does not recur; it simply continues to grow. Only the clinical symptoms recur.

REPORT OF CASES

CASE 1.—A man aged 24 was admitted to the Kennedy General Hospital in May 1944 for study. In October 1943 he had atypical pneumonia, which involved first the middle and then the upper and lower lobes of the right lung. The sputum contained pneumococci. A similar attack occurred in January 1944. In June 1944 cough and fever developed, and roentgen examination showed atelectasis of the lower lobe of the involved lung. On bronchoscopy an adenoma of the left

5. Maximow, A. A., and Bloom, W.: *A Textbook of Histology*, Philadelphia, W. B. Saunders Company, 1937, p. 35.

6. (a) Adams, W. E.; Steiner, P. E., and Block, R. G.: *Surgery* **11**:503, 1942.
(b) McDonald, J. R.; Moersch, H. J., and Tinney, W. S.: *J. Thoracic Surg.* **14**:445, 1945.

7. (a) Womach, N. A., and Graham, E. A.: *Arch. Path.* **26**:165, 1938;
(b) Graham and Womach.¹

primary bronchus was seen and the diagnosis was confirmed by biopsy of the tumor tissue. A left pneumonectomy was performed in September 1944. The patient's recovery was uneventful, and he was discharged from the hospital in December 1944.

Pathologic Examination.—In the left lung was a pedunculated tumor, which arose from the spur at the bifurcation of the left main bronchus. The tumor completely obliterated the upper bronchus. Mechanically it could at times just as well have obliterated the lower branch. The mucosa proximal and adjacent to the pedicle was slightly roughened and firm. Cross section of the tumor and part of the bronchus (fig. 1) showed the pedunculated tumor as it arose from the bronchus. It could have been removed through the bronchoscope, with relief of symptoms. Microscopic examination of the pedicle would not have revealed



Fig. 1.—Pedunculated tumor in case 1. The glands distal and proximal to the base of the peduncle contain tumor cells. $\times 45$.

the presence of tumor. Actually the tumor would have recurred. As may be seen in figure 1, the glandular structures both proximal and distal to the pedunculated tumor contained many tumor cells. Figure 2 A, a higher magnification of this area, illustrates the glandular structures and the tumor. It is true that the mucosa proximal to the pedicle was slightly roughened, but the mucosa distal appeared intact. This distal area of the tumor could not have been seen even on closer scrutiny after the bronchus had been opened. In the same way a cluster of glands behind the cartilage contained the tumor cells. Local removal of the tumor present, as well as of the tumors which would have arisen on recurrence of growth, could only have alleviated the condition, while during this period of temporization the tumor behind the bronchus would have invaded the surrounding tissue. Pneumonectomy would have become a necessity but under much less favorable conditions. Whether these different sites of tumor in these glandular

structures developed independently would be difficult to determine. Suffice it to say that a pedicle-free tumor does not preclude absence of tumor in the remaining portion of the bronchus. Small islands of cartilage and bone were seen in this tumor. Cases 1 and 2 (fig. 2 *A* and *B*) illustrate, perhaps better than any of the

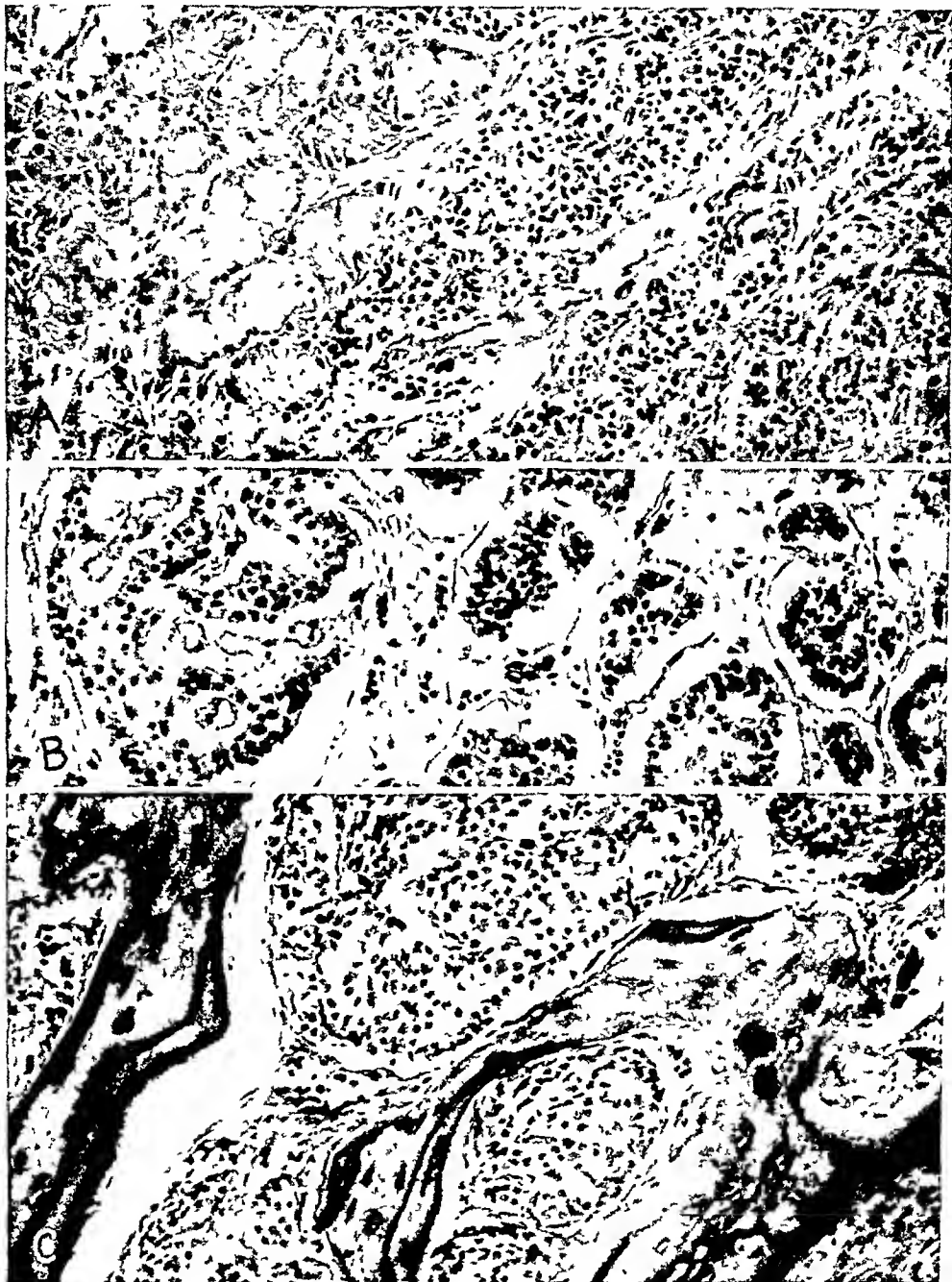


Fig. 2.—*A*, tumor cells distal to the pedunculated tumor shown in figure 1. $\times 200$. *B*, acinous arrangement of the tumor cells in case 2. *C*, bone occurring in bronchial adenoma in case 2. Bone is frequently found in specimens of bronchial adenoma. Abnormal bone formation in the lung is not unusual. $\times 200$.

others, the intimate relationship which exists between the tumor and the glandular structures of the bronchi without actual neoplastic invasion of the intervening tissue.

CASE 2.—A 22 year old man was admitted because of bronchial obstruction. An obstructing tumor was seen on bronchoscopy, but biopsy failed to reveal definite evidence of tumor. Excision of the lower lobe of the right lung was performed successfully, and the patient had an uneventful recovery.

Pathologic Examination.—A tumor measuring 3 by 2.5 cm. was found arising from the wall of the main lower branch bronchus. It compressed the bronchus, causing almost complete obliteration of the lumen. The mucosa of the bronchus was intact and grossly not invaded by tumor, although it was edematous and congested. On section the tumor was found to be formed by soft tissue and bone. In figure 2 *B* the acinous arrangement of the cells of this tumor is evident. The cells with the dark-staining nuclei are identical with those seen in figure 2 *A* of case 1. The pale cells are like the cells of the mucous glands and those seen in the isthmus of the glands. Note that when both types of cells are found together, the paler cells are always central and the small round cells peripheral. The nucleus of the pale type, has delicate chromatin markings and a small pale nucleolus. The cytoplasm is pale, taking neither the basophilic nor the acid stain too well. Mucicarmine stain is not more revealing. As can be clearly seen in figure 2 *B*, the acini sometimes contain ovoid bodies with deep-staining nuclei and a delicate but well outlined vacuoloid structure. These bodies do not resemble oncocytes.

There are, therefore, two types of epithelial cells in bronchial adenoma: the clear tumor cells which have a close resemblance to the secretory cells of the bronchial glands, and the deep-staining round cells almost identical with the basal cell layer. In different tumors and frequently in different areas of the same tumor, as in case 2 (fig. 2 *B* and *C*), one or the other type of cell predominates. The stroma is prominent and evenly distributed in figure 2 *B* (case 2) and scant in figure 2 *C* (same case).

Whenever there is predominance of the small round cells, there is lack of the tendency to acinous arrangement. This feature is clearly seen in figure 3 *A* of case 3.

CASE 3.—A 29 year old man had presented symptoms since August 1943. On the roentgenogram there was an infiltrative process, which cleared after penicillin therapy was begun, but there remained thin-walled cysts. Biopsy material removed elsewhere was diagnosed as infiltrative carcinoma. Tissue removed on bronchoscopy in this hospital was diagnosed here as bronchial adenoma. Pneumnectomy on the left side was performed, and the patient had an uneventful recovery.

Pathologic Examination.—The bronchus leading into the upper lateral portion of the lower lobe of the left lung was obliterated by a soft, pink tumor measuring 1.2 by 1 cm. Its base measured 0.9 cm. The tumor had invaded the anterolateral side of the bronchus, forming on the other, outer wall of the bronchus a small nodule, which measured 0.2 by 0.3 cm.

Microscopic Examination.—This tumor (fig. 3 *A*) had much less tendency toward acinous arrangement than the previous ones. The small round cells predominated. There was little stroma, and even more rare were pale cells such as those seen in case 2.

Though in general structure all these tumors were similar, and the same types of cells could be found in each one of them, it is evident that the number of cells of each type present varied considerably, and on biopsy they might even be confused, as in case 3, with undifferentiated cell carcinoma. (This tumor in a small fragment of tissue was diagnosed

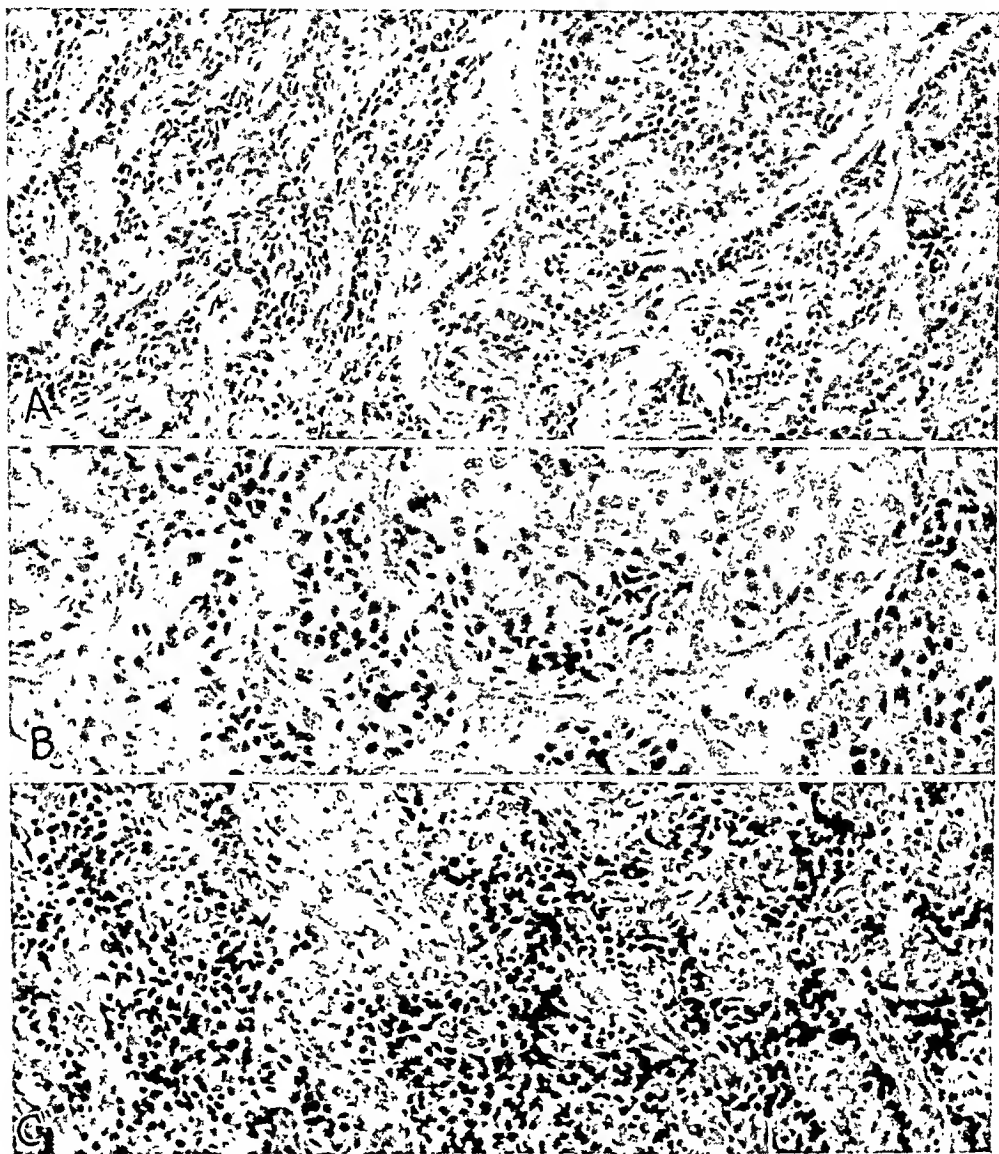


Fig. 3.—*A*, predominance of small round cells (case 3). $\times 200$. *B*, tendency toward acinous arrangement in an area where clear cells are found (case 4). *C*, predominance of small round cells (case 4). This predominance was noted throughout the tumor. There had been widespread invasion of the entire lobe.

by some of the most able pathologists as undifferentiated cell carcinoma and later, when the whole tumor was examined, as bronchial adenoma.)

CASE 4.—A man aged 51, who had no thoracic symptoms, was examined for an inguinal hernia, arthritis and chronic nephritis. During this work-up, a roent-

genogram of the chest was taken, which revealed atelectasis of the upper lobe of the right lung. Bronchoscopy revealed an obstruction of the bronchus of the right upper lobe. Biopsy material disclosed no evidence of tumor. In view of the obstruction and the patient's age it was considered that tumor was probably present in the bronchus of the upper lobe. During operation the patient ceased to breathe and could not be resuscitated. Postmortem examination showed that he had died of asphyxia secondary to an unusual amount of fairly adherent mucoid material in the bronchi and bronchioles of both lungs.

Pathologic Examination.—Almost the entire upper portion of the upper lobe of the right lung was found to be atelectatic and firm. When opened, the bronchi of this lobe showed evidence of a chronic inflammatory process. There were both longitudinal and cross striations in the mucosa, and the walls of the bronchi were thickened. One of the smaller bronchi was dilated and the mucosa destroyed, being replaced by a mass of dark mucoid material. In the immediate vicinity of this region the mucosa showed some papillary hyperplasia. The tissue surrounding this area was hard and infiltrated by tumor. The bronchi of the lower lobe were filled with a bloody mucoid exudate.

Microscopic Examination.—This tumor, as illustrated in figure 3B and C, contained both types of cells, but the small round cells predominated throughout. The tendency toward acinous arrangement had been almost completely lost except where the clear cells were found, as in figure 3B. Figure 3C illustrates the histopathologic character of the greater part of the tumor. In our opinion this is a so-called bronchial adenoma which has undergone malignant alteration, widely invading the upper lobe without, however, having produced metastases in the lymph nodes or other organs.

The last case is presented because it forms a composite picture of the 4 cases described in the preceding pages.

CASE 5.—A 35 year old woman had cough since the summer of 1944. A roentgenogram showed evidence of disease in the lower lobe of the right lung. On bronchoscopy a tumor was seen obstructing the bronchus of this lobe. Tissue removed from this tumor was diagnosed as adenoma. Pneumonectomy was done on the right side, and the patient had an uneventful postoperative recovery.

Pathologic Examination.—While in case 2 the tumor appeared to arise in the wall of the bronchus, leaving the mucosa intact, and in case 1 it was frankly pedunculated, this tumor resembled neither. It appeared to arise in the wall, penetrating the mucosa over an area measuring 1.4 by 0.9 cm. and completely obliterating the lower branch bronchus. The tumor likewise penetrated the outer wall of the bronchus into the lung parenchyma without, however, invading it, the tumor on this side being well encapsulated. Of all the specimens of bronchial adenoma, this one was by far the hardest, and this was found to be due to bone.

Microscopic Examination.—This tumor is of great interest because in it we found the different patterns described in the other 4 cases. A large part of the tumor was identical with that in case 1 (fig. 2B) and that in case 3 (fig. 3A) and another part again with that in case 2 (fig. 2C) except that here and there were less pale cells and fewer bone spaces with tumor. One area when seen isolated was diagnosed as undifferentiated cell carcinoma and resembled the small cell areas seen in case 4 (fig. 3B and C). On the other hand, this tumor was well delimited and did not invade the surrounding parenchyma.

Womach and Graham ^{7a} promulgated the theory that tumors of the type described arise from undeveloped bronchial buds. They expressed the belief that the presence of cartilage and bone in these tumors, though not always found,⁸ supports their hypothesis. On the other hand, Mallory ⁹ and Stout ⁸ have expressed the opinion that this is insufficient evidence. We tend to agree with them.

Of all the organs, the lung is the one exposed most to chronic infection with secondary inflammatory reaction. Also it is not unusual to find bone ¹⁰ arising at the extreme end of the cartilage crescents, especially if the inflammatory process has invaded the intercartilaginous spaces. Study of multiple sections of the upper ends of the larger bronchi in diseased lungs confirms this, and it is not unusual to find early calcific infiltration, osteoid tissue or bone. This is especially true when there is a long history of chronic infection.

Oncocytes, small round cells described by Hamperl,¹¹ unquestionably must be considered as a possible origin of these tumors. In case 1, as well as in case 2, several small cells with acidophilic cytoplasm were found, but they were not characteristic of the oncocytes, nor did they resemble the tumor cells. A study of the bronchial glands and the mucous glands in 50 cases of pulmonary diseases necessitating lobectomy showed that in about one fifth oncocytes could be found. In 1 case of cylindric bronchiectasis the mucous and the serous glands showed unusual secretory activity, and the mucoid material was packed with oncocytes, yet no bronchial adenoma could be demonstrated in this lobe. Several months later a specimen was sent to the laboratory which had been collected in the routine postoperative check-up of cases. Examination of the slide showed such a striking picture as to remind us of the previous case. When the slides of the previous case were reviewed, it was found that we were dealing with one and the same patient. These oncocytes do not have any resemblance to any of the cells found in any of our 5 cases of bronchial adenoma. When present in large number as in case 4, the cells were also found in the mucoid glands, yet these cells could not be confused with the tumor cells.

SUMMARY

Case 1 illustrates the inadequacy of local removal of pedunculated bronchial adenoma. This case also shows the intimate relationship of the bronchial adenoma to the mucous and the serous glands (fig. 2A).

8. Stout, A. P.: *Arch. Path.* **35**:803, 1943.

9. Mallory, P., cited by Stout.⁸

10. Wells, H. G., and Dunlap, C. E.: *Arch. Path.* **35**:420, 1943.

11. Hamperl, H.: *Virchows Arch. f. path. Anat.* **282**:724, 1931.

The presence of bone is described in 3 cases. It is considered insufficient evidence to support the hypothesis that such tumors originate from undeveloped bronchial buds.

All these tumors are considered to be "mixed", the two component cells being of epithelial origin.

The tumors described were found to have invasive tendencies and were considered as potentially malignant. In one, cancerous alteration had occurred. If only for this reason, radical surgical removal, when feasible, is advocated.

Oncocytes (Hamperl¹¹) have been found in 3 of these cases, but in none were they found so profusely as in the case of bronchiectasis in which no adenoma was found. These cells do not resemble tumor cells.

PRIMARY FIBROSARCOMA OF THE HEART WITH A VERTEBRAL METASTASIS

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ALTHOUGH primary tumor of the heart is rare in the experience of any one laboratory, the cases recorded in the literature form an impressive collection. Particularly rare is primary tumor of the heart with distant metastasis. Such a case is now reported, with a brief discussion of the clinical and pathologic aspects.

REPORT OF A CASE

A Russian-born, 47 year old housewife was first seen at the Peter Bent Brigham Hospital, Boston, on Aug. 17, 1944. The complaint was a steady non-radiating pain in the right scapular area with an ache in the right arm, numbness of the right palm, and stiffness and weakness of the fingers of the right hand. These symptoms had developed progressively since six months before admission when the patient had a brief illness characterized by cough with yellowish sputum, weakness, fatigability, and pains in the knees on walking. The cough persisted but became nonproductive. A lump had been removed from the left breast in 1916. No recurrences, metastases or other complications followed.

On examination the temperature was 100.2 F. (rectal), the pulse rate 112, the respiratory rate 20, and the blood pressure 100 systolic and 60 diastolic. The patient was a well developed, moderately obese, middle-aged woman. There was definite clubbing of fingers and toes. (The patient stated that the clubbing had been present as long as she could remember.) There was no tenderness of the spinal column. No abnormal masses were felt in the thorax, the abdomen or the extremities. The heart had a thrusting apical impulse just outside the left midclavicular line with a grade 2 apical systolic murmur and an exaggerated M₂. P₂ was greater than A₂. No diastolic murmurs were heard.

The red blood cell count was 5,200,000; the hemoglobin content, 12.8 Gm. per hundred cubic centimeters; the sedimentation rate (Wintrobe), uncorrected, 50 mm. in one hour. The white blood cell count was 11,800, with 80 per cent polymorphonuclears. Repeated blood cultures yielded no growth. The electrocardiograms showed only sinus tachycardia. A roentgenogram of the chest (August 19) revealed no cardiac enlargement, and no enlargement of the left auricle was seen by fluoroscopy. The lung markings were increased. A roentgen examination of the spinal column showed "slight scoliosis of the mid-thoracic part with convexity toward the right and a localized hypertrophic

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spurring anteriorly and laterally between the seventh and eighth thoracic vertebrae, with eburnation of the opposing margins. The intervertebral space is preserved."

The temperature ranged between 98.6 and 99.6, F. (oral); the pulse rate, between 80 and 100; the respiratory rate, between 20 and 30. On the fourteenth hospital day sulfadiazine therapy (6 Gm. a day) was begun and continued for fourteen days, without effect on the tachycardia or the fever. September 7, instillation of lipiodol, 40 per cent iodine, N. N. R., was attempted unsuccessfully. Following this, the temperature rose through the day to 103 F. and the pulse rate to 140, while the blood pressure fell to 90 systolic and 50 diastolic. That afternoon many rales were heard over both lung fields. The white cell count rose to 14,500. A roentgenogram of the chest (September 8) showed marked congestion about the hili, and a small amount of fluid at the base of the right lung. The heart was not enlarged. Over the following three days the patient improved. The rales cleared, and the temperature regained its previous level. A roentgenogram of the chest (September 18) showed a somewhat globular heart 4 per cent above average size by heart-weight ratio. There was less pulmonary congestion and no pleural fluid.

Second Admission (Nov. 6 to 22, 1944).—The backache had become more severe, keeping her awake nights, and the weakness of the right arm had increased. The numbness of the right palm now extended to include the second and fifth fingers. The nonproductive cough persisted. The patient had not apparently lost weight but appeared chronically ill. The fingers were perhaps more clubbed than before. The apical murmur was now louder.

The red blood cell count was 3,700,000, the hemoglobin content 10 Gm. per hundred cubic centimeters, the sedimentation rate 54 mm. in one hour and white blood cell count 11,000 with 85 per cent polymorphonuclears. Seven blood cultures yielded no growth. A roentgenogram of the chest (November 7) showed it to be enlarged 6 per cent above the average and globular in shape. The lungs showed coarse markings.

The pulse rate fluctuated between 80 and 100 per minute and the temperature between 98 and 100 F. (oral). The symptoms were unexplained and were poorly relieved by acetyl salicylic acid and codeine.

Third Admission (Jan. 11 to 21, 1945).—The patient was bedridden and was taking analgesics continuously for pain of the right shoulder and arm. She preferred an orthopneic position because the pain was less severe rather than because of dyspnea. She could scarcely move her right arm.

At this time her temperature was 101 F. (rectal), the pulse rate 128, the respiratory rate 26 and the blood pressure 118 systolic and 80 diastolic. The skin was pale, sweaty and cyanotic. There was marked atrophy of the interosseous and thenar muscles of the right hand. The heart was now enlarged to percussion, and both loud systolic and diastolic murmurs were heard at the apex. The liver was palpable and tender. There was moderate edema of the sacral area and of the legs. Rales were heard at the bases of the lungs. Clubbing of the fingers was now more marked on the right than on the left.

The hemoglobin was 9.6 Gm. per hundred cubic centimeters; the white blood cell count was 14,000, with 70 per cent polymorphonuclears. Repeated blood cultures yielded no growth. A roentgen examination of the spinal column showed "the transverse process of the first thoracic vertebra to be thin, hollow, as if filled with expanding tumor." The heart was enlarged 15 per cent. Pulmonary congestion was marked. A slight amount of fluid was present in both costophrenic

angles. The mediastinum appeared normal. The patient became progressively more cyanotic and dyspneic. Her temperature ranged between 98 and 102 F. and the pulse rate between 100 and 130. The sputum became bloody, and a roentgenogram of the chest (January 11) showed definite clouding of both lungs, with little aerated lung remaining. The white blood cell count rose to 41,900, with 94 per cent polymorphonuclears. Despite administration of digitalis and positive pressure oxygen, the patient died.

Necropsy (thirteen hours after death).—Permission to examine the head was not granted. Only the significant findings will be described at length. These were found in the heart and the vertebral column.

(a) Heart: The heart weighed 310 Gm. It was enlarged, moderately hypertrophied, and dilated, especially on the right side and in the left atrium. In the opened heart as seen from above, the lumen of the mitral valve appeared to be completely occluded by a pale yellow, firm, slightly lobulated, polypoid structure, firmly attached by a broad base to the auricular surface of the posterior leaflet and the adjacent atrial wall (fig. 1A). With some difficulty, the index finger could be passed from below between the tumor and the anterior leaflet of the valve. Passage from above was possible but was considerably more difficult. The over-all dimensions of the growth were: 4 cm. wide, 2.5 cm. high and approximately 3 cm. thick at the base. The external surface was alternately smooth, firm and yellow and pale pink, granular and friable. On cross section the growth fused imperceptibly with the endocardium and formed a coarse, pale gray, homogeneous, firm mass with poorly delineated, radially arranged bands separating adjacent peripheral lobulations.

The ventricular surfaces of the leaflets were smooth and glistening. The chordae tendineae were thickened but not fused or distorted. The papillary muscles were hypertrophied. The other valves of the heart showed no significant changes except for slight thickening, reduced translucency and fenestration of the pulmonary cusps. The endocardial surface of the left atrium was strikingly thickened and corrugated. Elsewhere it was thin, light gray and translucent. The myocardium of both the right and the left ventricle was hypertrophied. There were no other areas of tumor, thrombosis or scarring. The coronary vessels were pliable and of normal caliber throughout. There were no anomalies.

Small blocks of the tumor were immediately fixed in Zenker's fluid. The heart was fixed in 10 per cent solution of formaldehyde U. S. P. in toto, and several blocks were taken after fixation. The sections were stained in eosin and methylene blue, hematoxylin and eosin, phosphotungstic acid-hematoxylin, and aniline blue. They showed relatively acellular, irregularly arranged bundles and whorls of collagenous fibers with variably sized and shaped connective tissue cells with the appropriate stains (fig. 1B). Most of these cells appeared to be mature fibroblasts; others had plump spindle-shaped vesicular nuclei with abundant granular cytoplasm or delicate extracellular fibrillar projections. Still others were stellate or round with large hyperchromatic nuclei. There were also a few large tumor giant cells with amorphous dark nuclei. There was no evidence of cross striations, centrioles, intracytoplasmic fibrils or other staining qualities consistent with a tumor of muscle cell origin (Wolbach¹). There were no myxomatous or angiomatous features. On the basis of the foregoing observations the diagnosis of fibrosarcoma was made.

1. Wolbach, S. B.: Anat. Rec. 37:255, 1928.

The most active cell proliferation was seen at the periphery and the base of the growth. The center was composed of markedly hyalinized tissue. Invasion of the subendocardium was local and appreciable only microscopically. Most of



Fig. 1.—*A*, tumor seen from above through the opened left atrium. Note the strikingly occlusive character of the growth.

B, microscopic view of the primary tumor. Note the lobulation, the whorled character of the fascicles, the abundance of collagen and the wide variation of cell size and shape. (Eosin-methylene blue; $\times 143$.)

the tumor was seen to grow over a much thickened but otherwise undisturbed endocardium. The external surface was covered in part by a thin layer of platelets and fibrin. In a few places this covering and the subjacent part of the tumor were broken down.

(b) Vertebral Column: At the level of the first thoracic vertebra, directly beneath the bifurcation of the innominate artery, there was an ovoid smooth resilient mass measuring 5 cm. in length, 2.5 cm. in width and 1.5 cm. in height. The superior surface was encapsulated, smooth and free, while the base was intimately bound to the vertebral column. The tumor covered the latter and extended laterally to the right over the attachment of the second rib. On cross section the mass was seen to be a light gray homogeneous firm tissue enclosed by a fascial capsule. On pressure a small amount of dry friable material was expressed from the center. The tumor invaded and destroyed the underlying vertebral bodies and distinctly compressed the spinal nerve roots as they issued out of the spinal canal.

Blocks of the tumor were fixed and stained as described in an earlier paragraph. Microscopic examination showed it to be essentially identical with the one seen in the heart (fig. 2). The difference was in the much better preservation and more active proliferation of the cells of the former.

The seventh thoracic vertebra showed the characteristic lipping of hypertrophic arthritis. On gross cross section and on microscopic examination no tumor was found. Gross examination of the other thoracic, lumbar and sacral vertebrae failed to demonstrate any additional tumor.

(c) Lungs: The right lung weighed 640 and the left 500 Gm. Both were voluminous and practically airless. Numerous infarcts of varying size and age were found in both lungs. These were associated with thrombi of corresponding variation in age in the medium and small branches of the pulmonary artery. The source of the thrombi was apparently a thrombosed left popliteal vein. No thrombi were found in the major venous trunks and their tributaries, or in the chambers of the heart or in the veins of the right leg. The vessels of the arms were not examined.

There was marked thickening of the interlobular septums. The arterial branches were likewise thickened. Microscopically, there were marked fibrous thickening of the stroma and of the arterial tree, fresh and recent infarcts and fresh and older thrombi, the latter undergoing organization. The noninfarcted areas of the parenchyma showed alveoli filled with hemosiderin-laden macrophages, precipitated protein of edema, fibrin, a few leukocytes and many red blood cells. The capillaries were markedly distended. Aside from the infarctions, the changes found corresponded well to those of pulmonary fibrosis associated with severe mitral stenosis, described by Parker and Weiss.²

The other findings included moderate peripheral edema of the legs, clubbing of fingers and toes and atrophy of the small muscles of the right hand. The right and left pleural cavities contained approximately 200 and 100 cc. of a clear straw-colored fluid, respectively. The abdomen contained 150 cc. of a similar fluid. Approximately 15 to 20 cc. of the same fluid was present in the pericardial sac. There were no pleural, pericardial or peritoneal adhesions. All the structures of

2. Parker, F., and Weiss, S.: *Am. J. Path.* **12**:329, 1936.

the superior mediastinum were displaced slightly anteriorly but not laterally by the vertebral tumor. The large deep veins of the chest and the neck were unusually distended. Examinations of the other viscera did not reveal any additional tumor sites.

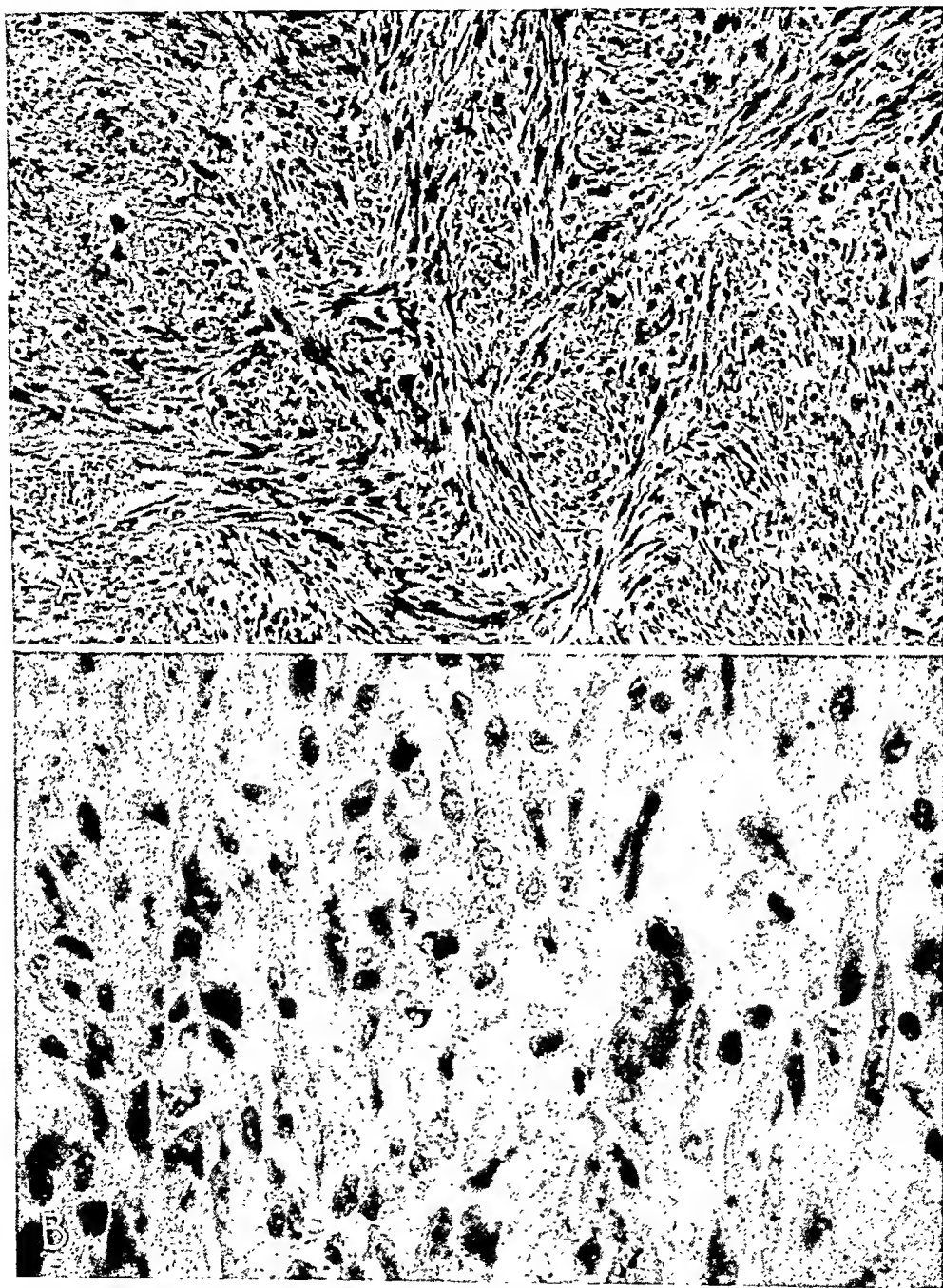


Fig. 2. *A*, microscopic view of the vertebral metastasis. The fundamental identity of pattern and cell type is well shown. (Phosphotungstic acid-hematoxylin; $\times 143$.)

B, microscopic view of the vertebral metastasis. Tumor giant cells, fibroglia fibrils and collagen are shown. (Eosin-methylene blue; $\times 800$.)

COMMENT

Clinical Aspects.—The antemortem diagnosis of tumor of the heart is rare. Mahaim³ in his remarkably comprehensive and critical review of the whole subject found 23 cases in which such a diagnosis had been made. In 5 more cases the diagnosis was suspected. In 20 of the 28 cases the tumor was secondary and in 8 it was primary. Two additional cases of metastatic tumor, reported by Hsiung and associates,⁴ should be added to this list. Only 3 established cases of primary intra-cardiac tumor recognized before death are known. These were reported by Popp⁵ Barnes, Beaver and Snell⁶ and Shelburne.⁷ It is of interest that in none of the 30 cases was the tumor benign.

In the opinion of the observers cited, the following findings are suggestive or diagnostic of a tumor of the heart:

- (1) Unexplained and intractable cardiac failure, which is often the first and the last.
- (2) Unexplained and sometimes inconstant changes in cardiac rhythm, sounds and size as judged by physical, roentgen and electrocardiographic examinations.
- (3) Development of a hemorrhagic pericardial effusion. (The presence of tumor cells in the fluid may confirm the diagnosis.)
- (4) Unexplained signs of obstruction of the cardiac blood flow or of the blood flow of the major thoracic vessels.
- (5) A specimen removed in arterial embolectomy which is shown on microscopic examination to be derived from a tumor in the heart.

In the case presented here the manifestations of the metastasis and the repeated pulmonary infarcts antedated and overshadowed the signs of the process in the heart. Yet there was progressive cardiac failure, a murmur which steadily became louder and a heart which steadily grew in size. One is tempted to speculate on the significance of the clubbing and of the patient's statement that it had been present as long as she could remember. The average duration of symptoms in most cases of tumor of the heart is under six months.⁸ In this case, the presenting symptoms began approximately one year before death, while the signs of cardiac failure appeared shortly before death.

3. Mahaim, I.: *Les tumeurs et les polypes du coeur: Etude anatomoclinique*, Paris, Masson & Cie, 1945.

4. Hsiung, J. C.; Szutu, C.; Hsieh, C. K., and Lieu, V. T.: *Chinese M. J.* 57:1, 1940.

5. Popp, L., cited by Mahaim.³

6. Barnes, A. R.; Beaver, D. C., and Snell, A. M.: *Am. Heart J.* 9:480, 1934.

7. Shelburne, S. A.: *Ann. Int. Med.* 9:340, 1935.

8. Ritchie, G.: *Am. J. Path.* 17:483, 1941.

In summary, while the diagnosis of tumor of the heart is theoretically feasible it has seldom been made. One reason for this may be the failure to consider such a possibility in the differential diagnosis. Mahaim³ rejected the generally held view that primary cardiac tumors are great rarities. He expressed the belief that were the incidence of such tumors based not on general autopsy figures but on those of patients with cardiac disease a more nearly true picture of the situation would be obtained. According to him, the diagnosis of such tumors is not merely of academic interest. A large number of them, i. e., the auricular polyps, may be amenable to surgical treatment in the near future.

Pathologic Aspects.—The general subject of cardiac tumors was reviewed by Yater,⁹ Lisa, Hirschhorn and Hart,¹⁰ and Ritchie.⁸ Primary tumors of the heart were reviewed by Haythorn, Ray and Wolff¹¹ and by Hamilton-Paterson and Castleden.¹² A summary of cases of primary sarcoma of the heart was made by Somolinos-D'Ardois.¹³ The most comprehensive clinicopathologic survey of the whole field of cardiac tumors, including those of the pericardium, was made by Mahaim³ in 1945. It is based on a critical review of the literature (1,298 references) and an unusually wide personal experience.

A few general conclusions of these and other observers must suffice.

Mahaim reviewed 413 cases of primary tumor of the heart—those in the literature and his own previously unreported cases. Excluded were 56 cases of organized thrombus or pseudomyxoma and a number of cases with inadequate or unreliable data. He pointed out that had the diagnosis of pseudomyxoma been based on adequate histologic study rather than on gross examination alone or on incomplete microscopic study, the total number of instances of primary tumor would undoubtedly be considerably increased. A table taken from his monograph is reproduced here because it summarizes well the classification and the distribution of the tumors.

As can be seen from the table, a classification based solely on anatomic grounds, i. e., polypoid and nonpolypoid tumors, is advanced. Despite the fact that this lumps together a wide variety of processes, the anatomic basis has diagnostic and probably therapeutic merit. The polypoid forms commonly produce signs of constant or transient interference with cardiac blood flow, and a number of the benign variants may some day be amenable to surgical removal. Thus he found 250

9. Yater, W. M.: Arch. Int. Med. **48**:627, 1931.

10. Lisa, J. R.; Hirschhorn, L., and Hart, C. A.: Arch. Int. Med. **67**:91, 1941.

11. Haythorn, S. R.; Ray, W. B., and Wolff, R. A.: Am. J. Path. **17**:261, 1941.

12. Hamilton-Paterson, J. L., and Castleden, L. I.: Brit. Heart J. **4**:103, 1942.

13. Somolinos-D'Ardois, G.: Arch. latino-am. de cardiol. y hemat. **10**:1, 1940.

instances of auricular polyp. In 174 of these the growth was occlusive, and in 70 per cent of the total number it was in the left atrium.

Out of a total of 87 cases of primary sarcoma of the heart, the auricles in 61 were involved, and the right auricle in 42 of these. In at least 42 of the total the tumor metastasized, mainly to the lungs. In none did it spread to the vertebrae. Our case brings the total to 88 and, to our knowledge, is the first instance of primary tumor of the heart involving vertebrae.

Mahaim's Table of Published Cases of Primary Tumor of the Heart and the Pericardium

	Polypoid	Nonpolypoid	Total
Heart			
Myxoma.....	82	23	105
Fibroma.....	8	29	37
Lipoma.....	4	10	14
Angioma (including lymphangioma).....	4	9	13
Rhabdomyoma.....	..	60	60
Celothelioma (mesothelioma) of node of Tawara	..	5	5
Miscellaneous (cysts and other benign tumors)...	..	8	8
Sarcoma.....	21	66	87
Total.....	119	210	329
Pericardium			
Fibroma.....	7		
Lipoma.....	3		
Angioma.....	10		
Miscellaneous (cysts).....	19		
Celothelloma (malignant).....	24		
Sarcoma.....	20		
Miscellaneous (malignant).....	1		
Total.....	84		84
			413

We believe that the tumor of the heart was primary, because metastatic tumor of the heart is multiple, involves the myocardium and is part of a widespread invasion of the body. It is unlikely that a metastasis derived from the vertebral tumor would have lodged in the left side of the heart without involvement of a lung. No cases of solitary cardiac metastasis of tumor are known (Ritchie⁸; Lisa and co-workers¹⁰; Burke¹⁴).

SUMMARY

A case of primary fibrosarcoma of the heart with a solitary metastasis, this involving the thoracic vertebrae, is presented. The clinical and pathologic aspects are briefly discussed.

14. Burke, E. M.: *Am. J. Cancer* 20:33, 1934.

HISTOLOGIC CHANGES OCCURRING IN THE HEMOPOIETIC ORGANS OF ALBINO RATS AFTER SINGLE INJECTIONS OF 2-CHLOROETHYL VESICANTS

A Quantitative Study

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THE PRINCIPAL object of this investigation was to assay by quantitative histologic methods the effects which intravenously injected sulfur and nitrogen mustard vesicants produced on the hemopoietic organs of the albino rat. The methods used had already been applied in a study of the hemopoietic organs of normal young adult rats.¹ Concerning sulfur mustard (mustard gas), it was known from clinical observations of victims of exposure² and from experimental study of laboratory animals³ that this vesicant produces leukopenia of the circulating blood and damages the lymphoid organs, the bone marrow³ and the intestinal mucosa.⁴ During World War II, under the direction of the chemical warfare services of Canada, Great Britain and the United States, extensive experimental studies were carried on with sulfur mustard and the newly developed toxic nitrogen mustards. A summary of the results and a discussion of the concepts of the chemical, biologic and therapeutic actions of the mustards have been made by Gilman and Phillips.⁵ The results described in the present paper are among those listed by name and date.

In brief, it has been shown that in laboratory animals in general the mustards caused transient hypoplasia of the lymphoid organs and

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4. (a) Lynch, V.; Smith, H. W., and Marshall, E. K., Jr.: *J. Pharmacol. & Exper. Therap.* **12**:265, 1918-1919. (b) Warthin, A. S., and Weller, C. V.: *J. Lab. & Clin. Med.* **4**:229 and (c) 265, 1919; (d) Winternitz, M. C.: *The Pathology of War Gas Poisoning*, New Haven, Conn., Yale University Press, 1920.

5. Gilman, A., and Phillips, F. S.: *Science* **103**:409, 1946.

of the bone marrow, accompanied by inhibition of mitosis in blast cells, which resulted in leukopenia of the circulating blood with marked initial lymphopenia, followed by neutropenia. The degenerative changes in the hemopoietic organs were accompanied by widespread systemic effects, among the most important of which were enteritis, pulmonary embarrassment, hypertrophy of the cortex of the adrenal gland and injury of the central nervous system. Certain of the nitrogen mustards produced more acute degenerative changes than others, and gradations of dose produced comparable degrees of degeneration. On the basis of the changes in the hemopoietic organs, which are notable sites of rapid cell growth, two of the agents, methyl-bis (2-chloroethyl) amine and tris (2-chloroethyl) amine, have been utilized in the treatment of persons suffering from neoplastic disease of the hemopoietic and other organs.⁶

The data presented in this paper are from reports presented to the Medical Division of the Chemical Warfare Service of the United States Army during 1945 and given in preliminary form before the American Association of Anatomists.⁷ They include: detailed quantitative histologic analyses of the thymus, the cervical lymph nodes, the spleen and the bone marrow of rats receiving intravenous injections of sulfur and nitrogen mustards in lethal (100 per cent) doses; a quantitative study of the white blood corpuscles of the circulating blood and of the adrenal glands; a qualitative study of the lungs, the liver, the kidneys, the hypophysis, the cerebrum, the cerebellum and the intestine; gravimetric measurements of the lymphoid organs and qualitative study of these organs and of the bone marrow of adrenalectomized rats receiving injections of tris (2-chloroethyl) amine, and an *in vitro* study of fragments of the thymus suspended in plasma and tris (2-chloroethyl) amine.

MATERIAL AND METHODS

Male albino rats of a Wistar strain, 60 to 70 days of age and weighing between 150 and 200 Gm., were used. In the biochemical laboratory, under the direction of Dr. Alfred Chanutin, the rats were anesthetized with pentobarbital sodium and given single injections of freshly prepared solutions of sulfur mustard (bis [2-chloroethyl] sulfide) and of the hydrochlorides of the following nitrogen mustards, respectively: ethyl-bis (2-chloroethyl) amine, methyl-bis (2-chloroethyl) amine and tris (2-chloroethyl) amine. The sulfur mustard was dissolved in thiodiglycol (2,2'-thiodiethanol) and the nitrogen mustards in saline solution. The jugular vein was dissected free, the agent injected in approximately 0.4 cc. of solvent and the incision closed. The solvent contained about 0.5 mg. of the sulfur mustard, and 1.0 mg. of each of the nitrogen mustards, per kilogram of body weight. These doses had been found by previous assay to be lethal for the rat. Control rats

6. (a) Rhoads, C. P.: *J. A. M. A.* **131**:656, 1946. (b) Goodman, L. S.; Wintrobe, M. M.; Dameshek, W.; Goodman, M. J.; Gilman, A., and McLennan, M. T.: *ibid.* **132**:126, 1946. (c) Jacobson, L. O.; Spurr, C. L.; Guzman-Barron, E. S.; Smith, T.; Lushbaugh, C., and Dick, G. F.: *ibid.* **132**:263, 1946.

7. Kindred, J. E.: *Anat. Rec.* **94**:474, 1946.

were anesthetized and saline solution (0.4 cc.) alone injected into the jugular vein. All rats were starved and killed one, two, three and four days after the injection. The rats were killed by exsanguination. At the time each rat was killed, blood was drawn from the jugular vein for counts of the white blood cells and for smears. The rats that received the injection of saline solution will be referred to as the control group; those that received the injection of sulfur mustard, as the SM group; those that received the injection of ethyl-bis (2-chloroethyl) amine, as the ECA group; those that received the injection of methyl-bis (2-chloroethyl) amine, as the MCA group, and those that received the injection of tris (2-chloroethyl) amine, as the TCA group.

The thymus, the anterior and posterior cervical lymph nodes, the spleen and the adrenal glands were removed and weighed. The organs of 4 or more rats in each group were fixed in Helly's fluid, embedded in paraffin, sectioned and stained either with Delafield's hematoxylin and eosin or Feulgen's or Mallory's stain. The right femur was split, and touch-smear preparations of the marrow were made and stained by the May-Grünwald method. The proximal end of the femur and its marrow were fixed in Helly's fluid.⁸ Later these femurs were decalcified in Perenyi's fluid, sectioned and stained as were the sections of the lymphoid organs. In addition, some of the sections were stained by the eosin-azure II-hematoxylin method of Maximow.

The volumes of the parts of organs were calculated by projecting images of serial sections on paper, outlining, cutting out and weighing the parts. The number of cells per unit volume was calculated by counting the cells per unit volume in one section from each organ or region of that organ under oil immersion magnification (1600 diameters). This method of calculation has been criticized by Abercrombie⁹ as being subject to error when extrapolations as regards distribution per larger volume are to be made from such samples. This criticism may be valid when cells in closely packed tissues are studied, but with the experimental materials referred to here, in which the cells are rather isolated, the error is small. Furthermore, calculations of the numbers of lymphocytes produced by the lymphoid organs of the rat made by the use of this method have been found to agree with counts of lymphocytes made directly from the thoracic duct.¹⁰

The percentage distributions of the several types of cells observed in the thymic cortex (unit volume, 70,000 cubic microns), the anterior cervical lymph nodes (unit volume, 90,000 cubic microns) and the spleen (unit volume, 70,000 cubic microns) were obtained by counting the cells of each specific type in each of these unit volumes. In these counts degenerated cells both inside and outside macrophages were included. The cells in mitosis in 25 unit volumes from the cortices of the thymus and the anterior cervical lymph nodes and from the lymphoid cords of the spleen, respectively, and in 10 unit volumes (1 unit volume per nodule) of the secondary lymphoid nodules of one anterior cervical lymph node and of the spleen of each rat were counted.

In regard to the bone marrow, the cell populations were counted in sections (unit volume, 50,000 cubic microns), but the percentage distributions of the types of cells were obtained from differential counts of 500 cells in stained smears from the marrow of each rat. Cells in mitosis were counted in 25 unit volumes

8. Helly's fluid is a modification of Zenker's solution in which, instead of glacial acetic acid, solution of formaldehyde U. S. P. is added in the concentration of 5 per cent.

9. Abercrombie, M.: *Anat. Rec.* **94**:239, 1946.

10. Reinhardt, W. O.: *Anat. Rec.* **94**:197, 1946.

per section of marrow from each rat, and the numbers of megakaryocytes observed in these volumes were recorded.

In the spleen, in addition to the quantitative study of the lymphoid tissue, the hemopoietic areas, the cells in mitosis in these areas and the megakaryocytes per 25 unit volumes were counted.

The quantitative data with their standard errors were listed in tables which included: (1) the average number of white blood corpuscles per cubic millimeter of circulating blood; (2) differential counts of the white blood corpuscles; (3) the average numbers of lymphocytes and neutrophilic granulocytes per cubic millimeter of circulating blood, calculated from (1) and (2); (4) the average weight of the lymphoid organs per hundred grams of body weight; (5) the average volumes of parts of the lymphoid organs and bone marrow; (6) the average percentage distributions of the several different types of cells per unit volume of tissues; (7) the average number of medium-sized lymphocytes in mitosis in a specific number of unit volumes of tissue from the lymphoid organs; and the average number of myeloid and erythroid cells in mitosis in the bone marrow.

These data were extensive, and although they are presented in detail in the Chemical Warfare Service reports, it was decided in the interest of brevity to present the changes in the number of cells and of cells in mitosis in tables which summarize the results and which are based on the weight of the organ, the volume of the specific part and the numbers and percentages of the different types of cells. The extent of variability in the gravimetric measurements and in the percentage incidence of certain of the cells of the thymus, the cervical lymph nodes, the spleen and the marrow are presented in graphic form as an aid to the understanding of the general trend of changes in these organs. Furthermore, only those organs and the parts of those organs which under normal conditions contribute significantly to the lymphocyte and granulocyte populations of the blood are so presented.

Photomicrographs were made of sections of the several organs of the controls and the rats given the vesicants. Because of the similarity of the morphologic changes resulting in the organs injured by the several agents, it has been felt that the changes which occurred in the rats receiving an injection of tris (2-chloroethyl) amine would illustrate the typical conditions. Hence the photographs presented are from one day control rats and rats which had received an injection of this vesicant.

In addition to these materials, the lymphoid organs and the bone marrow of adrenalectomized rats which had received injections of varying amounts of tris (2-chloroethyl) amine were studied quantitatively and qualitatively. Starved adrenalectomized rats served as controls for this group. Also, supravital examinations were made of samples of fragments of thymus suspended in a solution of plasma and tris (2-chloroethyl) amine, the latter in a concentration equivalent to that used for intravenous injection.

EXPERIMENTAL RESULTS

THE WEIGHTS OF THE RATS

The average body weight of the control group decreased significantly on the first day after the injection (7.0 ± 0.3 per cent), was significantly lower on the second day (13.0 ± 1.0 per cent) and remained at this level throughout the fourth day. All of the vesicant-poisoned groups showed significantly less decrease in average body weights on the first day than did the control group (SM, 4.2 ± 0.8 per cent; ECA, 2.1 ± 0.6 per cent; MCA, 2.7 ± 0.4 per cent; TCA, 4.7 ± 0.9 per cent). But at the end of the second day the weight of each of these groups except

the sulfur mustard group was significantly decreased from that of the first day. The weights of the nitrogen mustard groups remained at the same level as that of the control group on the remaining days of the period, but the weight of the sulfur mustard group was not as low as that of the control group until the fourth day. These data indicate that any losses of weight suffered by the rats given the vesicants were caused by acute inanition and not by the agents.

THE WHITE BLOOD CELLS OF THE CIRCULATING BLOOD

There were no significant changes of the average number of white blood cells of the circulating blood of the controls throughout the four day period (table 1, A). In all of the rats that received vesicants there was leukopenia initially. The average

TABLE 1.—*White Blood Corpuscles*

Rats were given single intravenous injections of saline solution, sulfur mustard (SM), ethyl-bis (2-chloroethyl) amine (ECA), methyl-bis (2-chloroethyl) amine (MCA) and tris (2-chloroethyl) amine (TCA), respectively. The average numbers of white blood corpuscles, lymphocytes and neutrophilic granulocytes per cubic millimeter of blood are recorded as found one, two, three and four days after the injection, respectively. WBC. means white blood corpuscles; L., lymphocytes; P., neutrophilic granulocytes; No., number of rats; v., standard error of mean.

A. Average Numbers of White Blood Corpuscles per Cubic Millimeter															
Days	Saline Solution			SM			ECA			MCA			TCA		
	No.	WBC.	v.	No.	WBC.	v.	No.	WBC.	v.	No.	WBC.	v.	No.	WBC.	v.
1	19	5,400	± 355	6	2,400	± 142*	6	2,100	± 500*	13	2,100	± 160*	6	1,830	± 246*
2	8	5,400	± 690	6	1,300	± 180†	10	1,125	± 165	10	1,150	± 200†	16	1,100	± 187†
3	14	4,950	± 507	6	3,600	± 1,010	4	1,800	± 500	8	850	± 118	6	1,000	± 158
4	13	6,400	± 510	7	4,400	± 860			10	650	± 58	7	1,675	± 240†
B. Average Numbers of Lymphocytes per Cubic Millimeter															
	No.	L.	v.	No.	L.	v.	No.	L.	v.	No.	L.	v.	No.	L.	v.
1	19	4,150	± 300	6	1,720	± 210*	6	1,110	± 420*	13	1,110	± 160*	6	455	± 84*
2	8	3,900	± 525	6	730	± 150†	10	310	± 270	10	630	± 167†	16	177	± 40†
3	14	3,400	± 390	6	1,500	± 750	4	325	± 62	8	690	± 108	6	520	± 115†
4	13	4,600	± 670	7	880	± 580			10	475	± 40	7	570	± 120
C. Average Numbers of Neutrophilic Granulocytes per Cubic Millimeter															
	No.	P.	v.	No.	P.	v.	No.	P.	v.	No.	P.	v.	No.	P.	v.
1	19	1,070	± 310	6	650	± 168	6	950	± 130	13	950	± 85	6	1,350	± 176
2	8	1,350	± 220	6	550	± 63	10	815	± 137	10	500	± 134†	16	920	± 152
3	13	1,430	± 500	6	2,000	± 700†	4	1,420	± 1,000	8	138	± 43†	6	450	± 270
4	13	1,600	± 150	7	3,450	± 500			10	155	± 30	8	1,040	± 83†

* The number is significantly different from the mean of the first day controls (saline solution).

† The number is significantly different from the mean of the preceding day.

count was reduced to 45 per cent of the control value in the SM group, to 39 per cent in the ECA and MCA groups and to 33 per cent in the TCA group. The leukopenia was more severe on the second day. On the third day, the control level was reached in the SM group, but the leukopenia continued throughout the remainder of the period in the nitrogen mustard groups. There appeared to be a slight alleviation of the leukopenia in the TCA group, but not in the MCA group, on the fourth day. No members of the ECA group survived beyond the third day.

The percentages of the several types of white blood cells were determined from differential counts of 100 cells of one smear of the blood of each animal (table 2). Only this number was counted because the distribution was so sparse in rats in which the total white blood cell count was less than 1,000 per cubic millimeter.

There were no significant changes of the percentage distributions of the myelocytes in any of the groups throughout the period. The juvenile neutrophils were

quite variable in their distribution but were most consistent in distribution in the control group. They decreased significantly in the MCA and TCA groups throughout the period. In the SM group they were significantly less numerous than in the control group until the fourth day.

The band neutrophils were practically absent from the blood of the controls throughout the period. In the poisoned rats their distribution was variable, and the only significant changes noted in a comparison of these and the control rats were increases in the TCA group on the first and fourth days and in the SM group on the third and fourth days. Thus the only groups to show a shift to the left in the neutrophil distribution at some time during the period were the TCA and SM groups.

TABLE 2.—*White Blood Corpuscles*

Rats were given single intravenous injections of saline solution and vesicants as listed, respectively. The average percentage distributions of juvenile (J-N), band (B-N) and segmented (S-N) neutrophilic granulocytes, lymphocytes (L.) and eosinophilic granulocytes (Eo.) are recorded as found in blood smears one, two, three and four days after injection, respectively; 100 cells were counted in one smear from each rat. Neg. means distribution less than 0.1 per cent; other abbreviations have the meanings given in table 1. Myeloblasts, myelocytes and monocytes are not included.

	No.	J-N. v.	B-N. v.	S-N. v.	L. v.	Eo. v.
A. One Day After Injection						
Saline.....	13	3.4 \pm 1.0	Neg.	16.5 \pm 2.0	76.0 \pm 2.5	1.4 \pm 0.3
SM.....	6	0.5 \pm 0.2*	0.5 \pm 0.2*	26.0 \pm 3.0	72.0 \pm 3.0	Neg.*
ECA.....	6	3.0 \pm 0.8	1.0 \pm 0.8	41.0 \pm 8.0*	53.0 \pm 9.5*	0*
MCA.....	6	2.0 \pm 0.7	1.3 \pm 0.7	42.0 \pm 5.0*	53.0 \pm 4.5*	0.6 \pm 0.1
TCA.....	9	1.0 \pm 0.5*	4.0 \pm 0.5*	69.0 \pm 7.7*	25.0 \pm 6.0*	0*
B. Two Days After Injection						
Saline.....	8	2.3 \pm 0.5	0.6 \pm 0.3	22.2 \pm 3.7	72.0 \pm 3.6	1.0 \pm 0.5
SM.....	6	0.5 \pm 0.3*	0.8 \pm 0.4	41.0 \pm 4.2*	57.0 \pm 4.6*	0.5 \pm 0.3
ECA.....	8	1.0 \pm 1.0	1.0 \pm 0.3	70.0 \pm 7.5*	27.0 \pm 7.0*	0
MCA.....	7	0.5 \pm 0.3*	0.5 \pm 0.2	43.0 \pm 8.4	55.0 \pm 8.0	1.0 \pm 0.3
TCA.....	15	Neg.*	1.0 \pm 0.3	82.0 \pm 2.9*	16.0 \pm 2.6*	Neg.
C. Three Days After Injection						
Saline.....	8	4.0 \pm 1.3	Neg.	25.0 \pm 1.8	68.0 \pm 2.4	1.0 \pm 0.8
SM.....	6	1.7 \pm 0.7*	3.5 \pm 1.1*	50.0 \pm 6.3*	42.0 \pm 6.7*	Neg.
ECA.....	4	6.0 \pm 2.4	4.0 \pm 2.6*	71.0 \pm 2.8*	18.0 \pm 2.6*	0.2 \pm 0.3
MCA.....	10	0.6 \pm 0.2*	0.7 \pm 0.4	15.0 \pm 4.2	82.0 \pm 4.5	Neg.
TCA.....	6	3.0 \pm 0.9	2.0 \pm 0.4*	40.0 \pm 3.4*	52.0 \pm 3.0*	Neg.
D. Four Days After Injection						
Saline.....	6	5.0 \pm 0.4	Neg.	20.0 \pm 1.0	72.0 \pm 3.0	1.3 \pm 0.3
SM.....	7	3.6 \pm 0.6	5.4 \pm 2.6	69.0 \pm 7.0*	20.0 \pm 6.4*	0*
ECA.....
MCA.....	10	2.0 \pm 0.4*	2.0 \pm 0.8*	20.0 \pm 3.7	73.0 \pm 4.5	0*
TCA.....	11	1.5 \pm 0.2*	3.3 \pm 1.9*	58.0 \pm 3.7*	34.0 \pm 3.7*	0.8 \pm 0.6

* The number is significantly different from the mean of the controls (saline solution).

The segmented or mature neutrophils showed no significant changes of distribution in the control group. There was initial relative increase of these cells in the nitrogen mustard groups. This initial increase persisted in the ECA and TCA groups but returned to the control level in the MCA group. The SM group did not show significant change until the second day, but the percentage continued to rise until the fourth day.

There were no significant changes of the percentage distributions of the lymphocytes throughout the period in the control group. There were initial significant decreases of percentage distributions of these cells in the nitrogen mustard groups. In the ECA and TCA groups these decreases continued throughout the period in the surviving rats (Note: there were no rats which survived the injection of ethyl-bis (2-chloroethyl) amine beyond the end of the third day.) In the MCA

group the percentage returned to the control level at the end of the second day and so continued until the end of the fourth day. In the SM group the only significant change throughout the period was a significant decrease at the end of the third day. The ECA and TCA groups varied the most consistently from the control group.

The eosinophilic granulocytes were distributed in very small numbers in the controls but were always present, whereas these cells were practically never present in the poisoned rats throughout the period. There were no significant changes in the incidence of the monocytes throughout the period in any of the groups, and basophilic granulocytes were so few that no changes could be observed.

There were no significant differences between the numbers of degenerated cells observed in the blood smears of the control rats and those of the poisoned rats.

In order to evaluate the trend of change in the absolute numbers of lymphocytes and neutrophilic granulocytes, the basic data of tables 1 A and table 2 were combined, and the results of these calculations are presented in table 1 B and C. There were no significant changes of the average number of lymphocytes per cubic millimeter in the control group throughout the period (table 1 B and fig. 1). In the poisoned groups there was initially marked lymphopenia. The relative decreases in the several groups in terms of the average of the first day control rats are shown in table 5. This lymphopenia was relatively greater than the leukopenia in all but the SM group. On the second day the lymphopenia became more severe in all groups. On the third day it remained at the lowest levels reached in the ECA and MCA groups, but the lymphocytes increased in number in the TCA and SM groups. On the fourth day there were significant decreases in the numbers of lymphocytes in the MCA and SM groups, but there was no change in the TCA group. Thus the counts of lymphocytes of the blood showed decreases proportionately greater than the decreases of the total leukocyte counts, and the initial leukopenia and lymphopenia were greatest in the TCA group and least in the SM group. The ECA group showed the greatest decrease after the first day, the MCA group showed no indication of regeneration of lymphocytes, while in the TCA and SM groups this was initiated on the third day, but showed relapse in the SM group on the fourth day. The amelioration of the leukopenia by increase of lymphocytes was characteristic of the TCA and SM groups.

In the control group there were no significant changes of the neutrophil count throughout the period (table 1 C and fig. 10). In the poisoned groups there were no significant changes of the neutrophil counts on the first day. On the second day the only group to show significant change was the MCA group, in which there was significant neutropenia. On the third day there was significant neutrophilia in the ECA and SM groups (70 and 260 per cent neutrophils, respectively), which was paralleled in the SM group by significant increase in the total leukocyte count. The MCA and TCA groups showed significant decreases in the neutrophils of 72 and 51 per cent, respectively. In the MCA group this decrease resulted in continued decrease of the total leukocyte count, but in the TCA group this decrease was neutralized by marked increase in the number of lymphocytes, so that the total leukocyte count did not change. On the fourth day, the neutrophils remained at the low level of the third day in the MCA group, but their number increased in the TCA and SM groups by 130 and 50 per cent, respectively. In these groups the rises accounted for the increases in the total leukocyte counts of the fourth day.

In brief, the data show that the number of neutrophils of the circulating blood was not changed in the ECA group but was significantly reduced in the MCA and in the TCA group; in the TCA group, however, it showed recovery. In the SM group there was neutrophilia on the third and fourth days. In contrast with the

neutrophils, the lymphocytes were markedly reduced in number, so that the leukopenia observed in the SM, ECA and TCA groups was caused by the lymphopenia, and that in the MCA group by both lymphopenia and neutropenia, with the lymphopenia having the greatest influence on the total leukocyte count.

THE THYMUS

The average relative weight of the thymus fluctuated in the control group throughout the period (fig. 1). It was significantly lower at the end of the second and fourth days than at the end of the first day. Despite these fluctuations, the average relative thymic weights of the nitrogen mustard groups were significantly less than that of the control group at all times. The average thymic weight of the sulfur mustard group was not less than that of the control group until the end of the third and fourth days, and at the end of the fourth day the value was the lowest reached in any of the groups. In the MCA and TCA groups the weight decreased throughout the end of the third day and remained at the lowest level through the fourth day. The relative decreases in the average thymic weights of the poisoned groups as compared with the average of the control group at the end of the first day are shown in table 5.

There were no significant changes of the average relative volume of the cortex of the thymus in the control group during the first three days after injection (fig. 1), but at the end of the fourth day it was significantly smaller than on the preceding days (85 per cent of the volume of the first day). Among the poisoned groups the volumes were significantly less in the nitrogen mustard groups than in the control group at the end of the first day (ECA and MCA, 86 per cent of the control; TCA, 65 per cent). In the MCA and TCA groups the volumes remained less than the control throughout the four day period. In the ECA group the volume was variable and showed return to control level at the end of the second day, but was less than the control on the third day. In the SM group there was no decrease in relative volume until the end of the second day (70 per cent of the control), and it remained at the lower level throughout the period.

The combination of dense cortex and light medulla with sharp demarcation between these parts as seen under low magnification, a condition which is characteristic of the normal thymus of the rat of this age, was present in the thymuses of the controls throughout the period. Figure 2*a* shows the characteristic morphologic aspect of the thymic lobules as seen in the controls. In all of the poisoned rats there were no gross alterations of this pattern on the first day (fig. 2*b*), but in the cortices of all there were light spots ("pits") where macrophages were active in areas of cell degeneration (fig. 3*b*). On the second day, only in the SM group was the pattern unchanged; in the ECA and MCA groups, the cortex was thin and loose, and there was only a faint irregular line of demarcation between cortex and medulla; in the TCA group, there was no line of demarcation between these regions, and small lymphocytes were scattered irregularly through both (figs. 2*c* and 3*c*). On the third day there was no line of demarcation between cortex and medulla in any of the nitrogen mustard groups (figs. 2*d* and 3*d*). There were more small lymphocytes in the medulla than in the cortex. On the fourth day the same pattern was characteristic of all groups, including the SM group (figs. 2*e* and 3*e*).

The cortex of the thymus is composed of a reticular framework in which are found large, medium-sized and small lymphocytes, reticulum cells and a few macrophages. A blood capillary plexus and a lymphatic network are also present, ramifying between the cells. The medium-sized lymphocytes are active mitotically,

and it is believed that they form an important center of the production of lymphocytes for the circulating blood of the rat.¹ Normally there is little cell degeneration. Other investigators have observed that following treatment with the vesicants there occurred destruction of lymphocytes, decrease of mitotic cells and shrinkage of the cortex.⁵ No quantitative study has been made by these investigators. The present study is an attempt to measure these changes in the

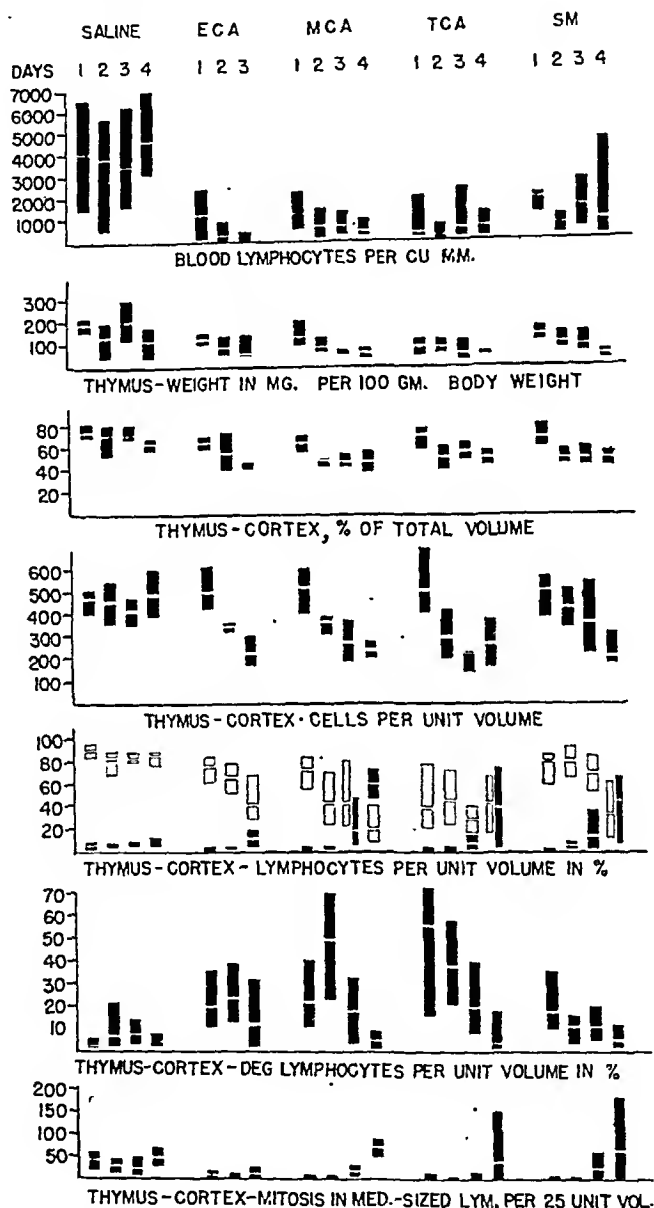


Fig. 1.—Block diagrams showing distributions of the lymphocytes of the circulating blood, the weight of the thymus, the volume of the thymic cortex, and distributions of the lymphocytes of the cortex of rats one to four days after single injections of saline solution and nitrogen and sulfur mustards. A white line across a black column indicates the mean, the upper end of the column the maximum and the lower end the minimum value, except in the first section on the lymphocytes of the thymic cortex, where the black columns represent distributions of the medium-sized lymphocytes, and the white columns those of the small lymphocytes. In the white columns the space across the column is the mean.

thymuses of vesicant-poisoned rats. It was thought that these quantitative changes could be demonstrated most favorably by extrapolation, the raw data on the relative weight of the thymus, the relative volume of the cortex and the incidence of the cells and of the cells in mitosis as they occurred in volumes of specific size being

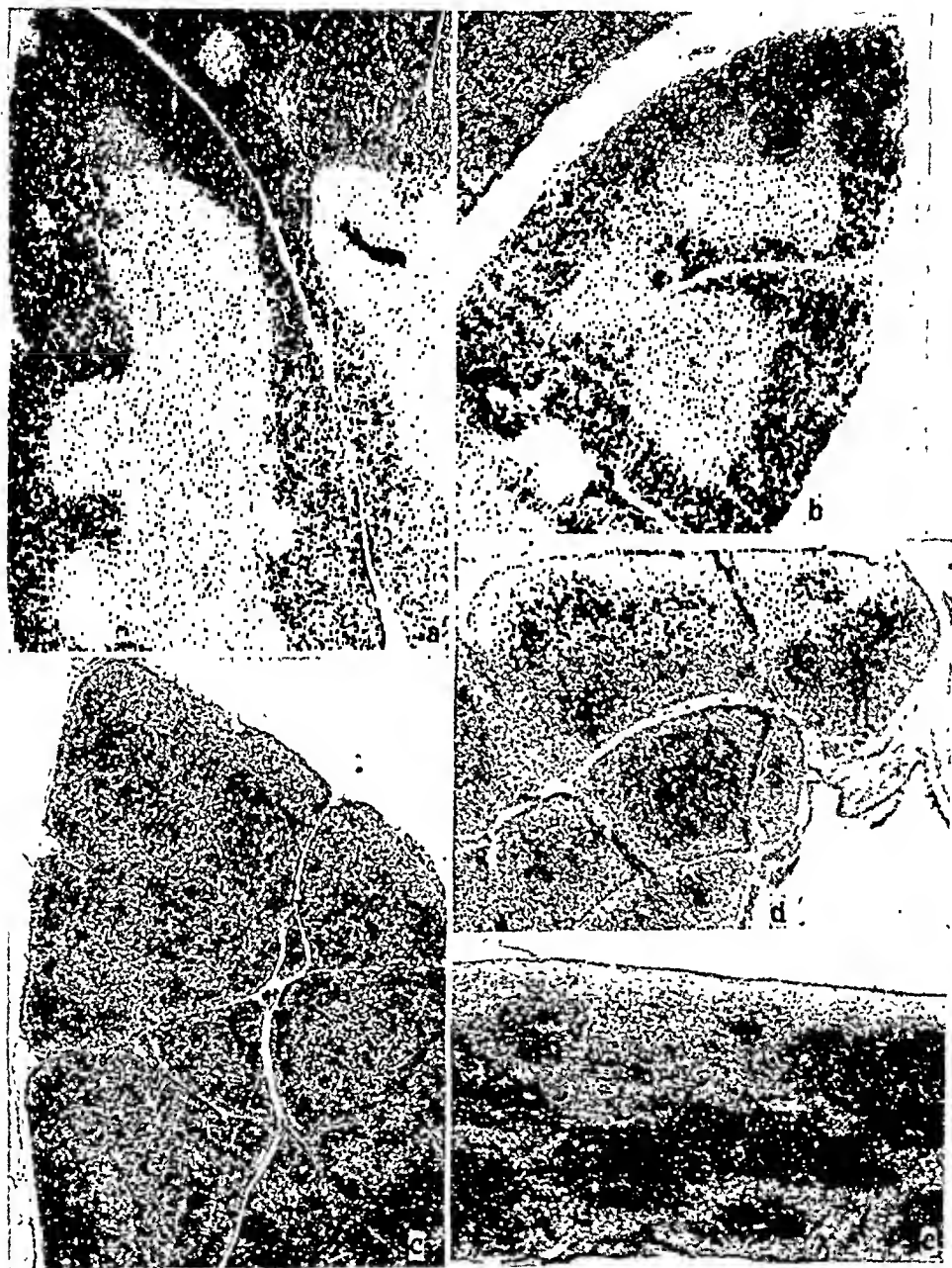


Fig. 2.—Photomicrographs of lobules of the thymus; hematoxylin and eosin; sections 7 microns thick; $\times 43$. (a) Control one day after injection of saline solution. (b, c, d and e) One day, two days, three days and four days, respectively, after injection of tris (2-chloroethyl) amine.

used (fig. 1). It is acknowledged that this method is not mathematically absolute, but it is felt that the results obtained by its use give a more objective view of the morphologic changes than would description based on qualitative findings alone.

All of the raw data on cell distributions are not included in this paper, because it was thought that the larger view presented by extrapolation would be sufficient to show the trends of change. From the combined data on weight of thymus, volume of cortex and incidence of cells and of cells in mitosis (fig. 1), the data in table 3 have been calculated. These data show the percentage distributions of the

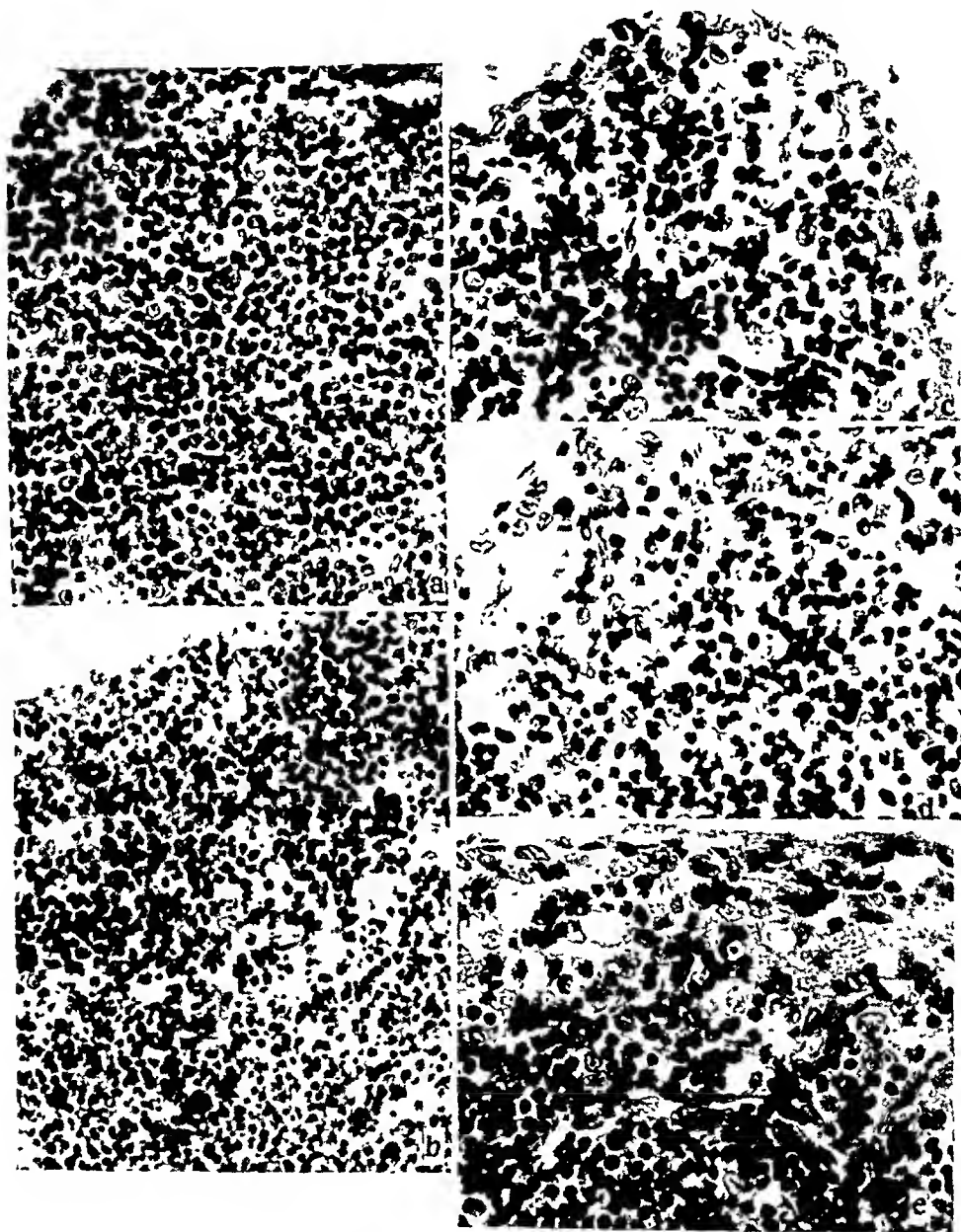


Fig. 3.—Photomicrographs of thymic cortex from sections shown in figure 2; $\times 201.5$. (a) Control one day after injection of saline solution. (b, c, d and e) One day, two days, three days and four days, respectively, after injection of tris (2-chloroethyl) amine.

several types of cells and the incidence of mitosis in the cells in the thymic cortex per hundred grams of body weight on four successive days following the injection of saline solution and of the vesicants. Any value which is distinguished by an

asterisk deviated significantly from the control value of the first day, and any value which is marked by a dagger is significantly different from the value of the preceding day in the group.

In the control group there were fluctuations in the total cell population of the thymus and a tendency toward reduction in number, so that on the fourth day the population was significantly less than it was on the first day (table 3 A). This reduction is believed to reflect the effects of acute inanition. In all of the poisoned groups there was significant hypoplasia of cells on the first day. On the second day the hypoplasia was more severe, and it continued in severity on the third day in all except the SM group. On the fourth day there were no significant changes from the third day except in the SM group, in which there was a marked decrease.

In the control group the large and the small lymphocytes of the thymus were significantly reduced in number as a result of inanition (table 3 B and D). However, the reductions which occurred in the controls were negligible when contrasted with the hypoplasia of all types of lymphocytes which took place on the first day in all of the poisoned groups. The large lymphocytes never recovered from the initial hypoplasia in any of the poisoned groups, although there was a tendency toward regeneration on the third day in the SM and MCA groups and on the fourth day in the TCA group. The medium-sized lymphocytes began to regenerate in the MCA group on the third day, and there was a trend toward regeneration in the SM group (table 3 C). In all of the poisoned groups the numbers of medium-sized lymphocytes were significantly greater on the fourth day than on the first.

The initial hypoplasia of the small lymphocytes was not relatively as great in any of the groups as were the reductions in the numbers of the larger lymphocytes, but in contrast with the tendency toward regeneration of the larger forms, the small lymphocytes continued to decrease throughout the four day period (table 3 D). Tris (2-chloroethyl) amine seemed to have the greatest hypoplastic effect on the small lymphocytes.

Coincident with the decrease in the numbers of medium-sized lymphocytes there was initial significant decrease in the numbers of medium-sized lymphocytes in mitosis (table 3 F). This was greatest in the TCA group and least in the MCA group. However, the decrease in the numbers of cells in mitosis was relatively greater than the decrease in the numbers of medium-sized lymphocytes; hence it is believed that the vesicants not only destroy lymphocytes but inhibit mitosis. This inhibition increased in severity in the TCA and MCA groups on the second day but did not change in the other groups. It seemed to have ceased in the MCA and in some members of the SM group on the third day, and there was evidence of resumption of mitotic activity. This occurred in the TCA group on the fourth day. The SM group showed the greatest variation of any of the groups. It is believed that the tendency for increase in the number of medium-sized lymphocytes and in the number of these cells in mitosis is an indication of a trend toward regeneration of the thymus and renewed production of lymphocytes destined to be used by the blood.

Degenerated lymphocytes were few, variable in number and usually isolated in the controls (table 3 E and fig. 1). In the nitrogen mustard groups there were initially significantly more degenerated lymphocytes than in the control group, but in the sulfur mustard group, although in some of the rats the thymus contained large numbers of degenerated lymphocytes, there was no uniform extensive degeneration such as occurred in the nitrogen mustard groups. These degenerated lymphocytes were largely included within macrophages of reticulum cell origin and were more numerous at the margin of the cortex than in the deeper regions. Also they tended to accumulate around blood vessels as if the reticulum cells which

ingested them had arisen from modification of periarterial reticulum cells. In the nuclei of these degenerated cells the chromatin was broken up into small pyknotic droplets which gave a positive reaction for desoxyribonucleic acid in Feulgen-stained preparations.

TABLE 3.—*The Thymus*

Rats received single injections of saline solution and vesicants as listed, respectively. The average numbers of cells of different types ($\times 10^3$) and of medium-sized lymphocytes in mitosis ($\times 10^3$) in the cortex per hundred grams of body weight are recorded as found one, two, three and four days after injection, respectively. The number of organs used per day were: saline control, 6; SM, 5, 5, 4, 4; ECA, 6, 6, 4; MCA, 6, 5, 6, 9; TCA, 6. The distributions of cells were calculated from the data in figure 1 and more detailed unlisted data on large lymphocytes, reticulum cells and macrophages. Each average is followed by its standard error.

Days	Saline Solution	SM	ECA	MCA	TCA
A. Cells of All Types					
1	94,600 \pm 5,850	57,000 \pm 8,600*	59,000 \pm 4,500*	58,700 \pm 7,600	56,900 \pm 12,300*
2	60,000 \pm 13,800	30,000 \pm 2,800†	24,500 \pm 3,140†	21,000 \pm 3,460†	20,000 \pm 3,900†
3	77,000 \pm 8,550	28,000 \pm 10,300	7,400 \pm 2,280†	10,200 \pm 935†	6,500 \pm 1,070†
4	58,000 \pm 10,300	7,000 \pm 1,680	9,300 \pm 850	11,200 \pm 1,680
B. Large Lymphocytes					
1	460 \pm 45	35 \pm 18*	90 \pm 34*	122 \pm 48	16 \pm 16*
2	400 \pm 137	80 \pm 29	113 \pm 61	33 \pm 19†	50 \pm 28
3	570 \pm 112	143 \pm 70	29 \pm 4	156 \pm 53†	8 \pm 8
4	200 \pm 61†	26 \pm 26†	116 \pm 27	131 \pm 48†
C. Medium-Sized Lymphocytes					
1	5,000 \pm 770	800 \pm 375*	670 \pm 314*	655 \pm 221*	633 \pm 238*
2	3,520 \pm 1,000	910 \pm 351	562 \pm 116	360 \pm 123	335 \pm 100
3	4,800 \pm 530	3,050 \pm 1,280	774 \pm 165	2,450 \pm 1,000†	473 \pm 153
4	3,870 \pm 730	2,345 \pm 1,230	6,000 \pm 640†	4,210 \pm 1,520†
D. Small Lymphocytes					
1	82,000 \pm 6,000	45,000 \pm 8,452*	43,000 \pm 2,700*	44,000 \pm 8,000*	19,400 \pm 4,500*
2	51,500 \pm 12,300	24,000 \pm 3,015	14,700 \pm 2,120†	8,213 \pm 2,130†	9,800 \pm 4,000
3	62,600 \pm 6,200	20,000 \pm 8,000	2,930 \pm 650†	3,600 \pm 1,150†	1,180 \pm 1,360†
4	51,200 \pm 9,100	3,100 \pm 1,000†	1,730 \pm 320†	5,600 \pm 1,520†
E. Degenerated Lymphocytes					
1	2,300 \pm 480	9,000 \pm 2,680*	12,600 \pm 3,560*	11,500 \pm 1,530*	34,100 \pm 11,200*
2	2,850 \pm 585	3,000 \pm 545†	5,800 \pm 1,740	10,600 \pm 3,000	6,775 \pm 1,070†
3	6,030 \pm 1,820	2,700 \pm 880	707 \pm 310†	1,911 \pm 315†	1,250 \pm 342†
4	1,670 \pm 555	281 \pm 120†	255 \pm 62†	400 \pm 183†
F. Medium-Sized Lymphocytes in Mitosis					
1	3,340 \pm 620	150 \pm 62*	250 \pm 125*	800 \pm 28*	66 \pm 66*
2	2,120 \pm 460	70 \pm 24	100 \pm 32	33 \pm 16†	13 \pm 8
3	1,620 \pm 490	550 \pm 410	150 \pm 60	218 \pm 83†	44 \pm 27
4	2,200 \pm 460	855 \pm 675	1,370 \pm 351†	1,140 \pm 600
G. Reticulum Cells					
1	2,460 \pm 270	950 \pm 315*	1,100 \pm 235*	964 \pm 296*	600 \pm 282*
2	2,850 \pm 585	1,651 \pm 615	2,756 \pm 710	1,150 \pm 350	2,710 \pm 635†
3	1,570 \pm 215	800 \pm 129	2,840 \pm 1,320	1,870 \pm 730	1,776 \pm 445
4	1,300 \pm 314	1,000 \pm 310	1,188 \pm 128	1,170 \pm 130
H. Macrophages					
1	50 \pm 25	930 \pm 235*	900 \pm 710*	1,060 \pm 167*	2,156 \pm 600*
2	180 \pm 72	140 \pm 31†	505 \pm 100	100 \pm 40†	423 \pm 240†
3	80 \pm 36	500 \pm 71†	170 \pm 61†	150 \pm 66	1,160 \pm 238
4	170 \pm 74	102 \pm 16†	250 \pm 60	280 \pm 107†

* The number is significantly different from the mean of the first day controls (saline solution).

† The number is significantly different from the mean of the preceding day.

When contrasts were made between the numbers of degenerated lymphocytes and the reductions of the lymphocyte populations as a whole in the poisoned rats, it was found that only a small percentage of the loss of lymphocytes could be accounted for by the degeneration. Hence it is believed that the greater part of the loss of lymphocytes was caused by migration of lymphocytes. This activity would

be related directly to the inhibition of mitosis of medium-sized lymphocytes and the consequent loss of lymphocytes which normally would enter the circulation. The unaffected lymphocytes would migrate to the blood stream in an attempt to alleviate the effects of decreased production. This view was partly substantiated by the observation that when regeneration began in the thymus there was a certain degree of alleviation of the lymphopenia of the blood. This correlation was not so great as the correlation between the destruction of medium-sized lymphocytes and the inhibition of their mitosis and the initial lymphopenia of the blood.

After the initial increase of the numbers of degenerated thymic lymphocytes of the poisoned groups there was significant decrease of the numbers of degenerated cells on the second day in all except the MCA group. Methyl-bis (2-chloroethyl) amine had apparently a more lingering toxic effect than did the other agents. After the second day the numbers of degenerated lymphocytes of all groups were negligible. Since the numbers of small lymphocytes decreased at this time, it is evident that these lymphocytes were not lost in the thymus by degeneration. As pointed out in the foregoing paragraph, it is more probable that the majority migrated and that those which remained grew into medium-sized lymphocytes, thus accounting for the increase in the numbers of these forms on the fourth day.

Normally, the reticulum cells of the thymic cortex show little phagocytic activity, and it can be seen at once that the macrophages of reticulum cell origin in the control group are negligible in distribution (table 3H). However, in all of the poisoned groups there were marked initial significant hyperplasia of the macrophages and significant hypoplasia of the unmodified reticulum cells (table 3G). The macrophages contained degenerated lymphocytes. Beginning on the second day, there were significant reductions in the macrophage populations and a trend toward restoration of the numbers of unmodified reticulum cells. No doubt some of these macrophages perished as a result of their unusual phagocytic activity, but the majority apparently became inactive after having digested the degenerated lymphocytes and appeared morphologically as unmodified reticulum cells. The reticulum cells were apparently not injured directly by the agents, and such loss of reticulum cells as occurred was secondary to their reaction to the unusual function of phagocytosis.

It is interesting to note that as the lymphocytes were being digested by the macrophages the chromatin of the nuclei of the ingested cells retained its specific reaction to Feulgen's reagent, because of the presence of desoxyribonucleic acid. After digestion there were no traces of desoxyribonucleic acid in the cytoplasm of the reticulum cells. Since the number of degenerated lymphocytes within macrophages decreased markedly in all groups except the MCA group on the second day, it is believed that this decrease may be used as evidence for the conclusion that it takes twenty-four hours for a macrophage to digest an injured lymphocyte. This view of course is based on circumstantial evidence, but it coincides with the observation of Clark and Clark¹¹ on the time required for a macrophage to digest a nucleated erythroid cell in the tadpole's tail. I have used this time relation in calculating the production-destruction cycle of the lymphocytes in the lymphoid organs of normal rats.¹

In brief, the quantitative data presented appear to indicate that the vesicants produce marked histologic changes in the thymus as a result of their destructive action on the larger lymphocytes and their inhibitive action on the mitotic mechanism of the medium-sized lymphocytes. The reticulum cells are not injured directly by the agents, but many react as macrophages and ingest and digest the

11. Clark, E. R., and Clark, E. L.: *Am. J. Anat.* **46**:91, 1930.

degenerated lymphocytes. Some of the reticulum cells perish as a result of this activity. The toxic effects of the agents apparently last for two days, after which there is regeneration of the larger lymphocytes and resumption of mitosis. The decrease in the weight of the thymus is thought to be largely due not to the degeneration of the cells but to migration of small lymphocytes. These lymphocytes which normally offer a large margin of safety in the supply-demand relation between the thymus and the circulating blood are drained from the thymus when the normal proliferative supply is cut off and thereby prevent total lymphopenia of the blood during the height of the toxic action of the agents.

THE CERVICAL LYMPH NODES

The average relative weight of the cervical lymph nodes was significantly lower on the second than on the first day in the control group, but on the third and fourth days there were no significant differences from the weight of the first day (fig. 4). It appears that acute inanition had a temporary depressing effect on these nodes. Among the vesicant-poisoned groups there was no initial change in the relative weight of the lymph nodes in the ECA group, but in the other groups the weights were significantly lower than that of the control (table 5). On the second day the weights in all of the vesicant-injured groups were significantly lower than that in the control group; furthermore, in the ECA and MCA groups the weights were lower than on the first day in these groups; in the TCA group the weight was significantly higher, and there was no change in the SM group (fig. 4). There were no further significant changes in the ECA, MCA and SM groups throughout the period, but in the TCA group there was a significant decrease on the third day which continued into the fourth day (table 5). In brief, there were significant decreases in the weights of the cervical lymph nodes in all groups after the first day.

Normally the cortex of the lymph node outside of the nodules produces only enough lymphocytes to maintain local growth and replace degenerated cells.¹ There is normally little degeneration, and the lymphocytes, predominantly of the small variety, are closely packed into the reticular stroma along with a small number of larger lymphocytes and reticulum cells. Cells in mitosis are few. These conditions were present in the cervical lymph nodes of the controls (fig. 5 *a*). Comparative quantitative study of the relative volumes of the cortices, the numbers of cells per unit volume, the distributions of the different types of cells and the numbers of cells in mitosis showed that in the samples from the poisoned groups the volume of the cortex shrank in proportion to the shrinkage of the whole node (fig. 5 *b* to *e*). There were marked hypoplasia of the lymphocytes, marked increase in degenerated lymphocytes, particularly around the periphery, and marked initial increase in macrophages which ingested degenerated lymphocytes. There was no generalized necrosis, and little fibrosis; the latter when present was near the hilus. The loss of lymphocytes could not be accounted for by the number of degenerated lymphocytes. It is believed that the great majority of lymphocytes which were lost from the lymph nodes entered the blood stream and with the lymphocytes from the thymus prevented complete lymphopenia of the circulating blood. After three days the degenerated lymphocytes had practically disappeared from the cortex and there were indications of regeneration and maintenance of the lymphoid character, although the nodes were smaller than normal. This part of the node is viewed as an accessory that prevents lymphopenia when the normal sources of the lymphocytes of the circulating blood are cut off. The quantitative data used as bases for the foregoing description are on file in this laboratory and in the files of the Medical Division of the Chemical War Service.

There was no significant change in the average relative volume of the secondary lymphoid nodules of samples from the anterior cervical lymph nodes of the control group throughout the period (fig. 4). In the poisoned groups there were no significant differences from the control group until the second day. At this time in the ECA, TCA and SM groups the relative volumes of the nodules were significantly lower than that of the control group. In the MCA group the relative volume did not change throughout the period. In the TCA group the relative vol-

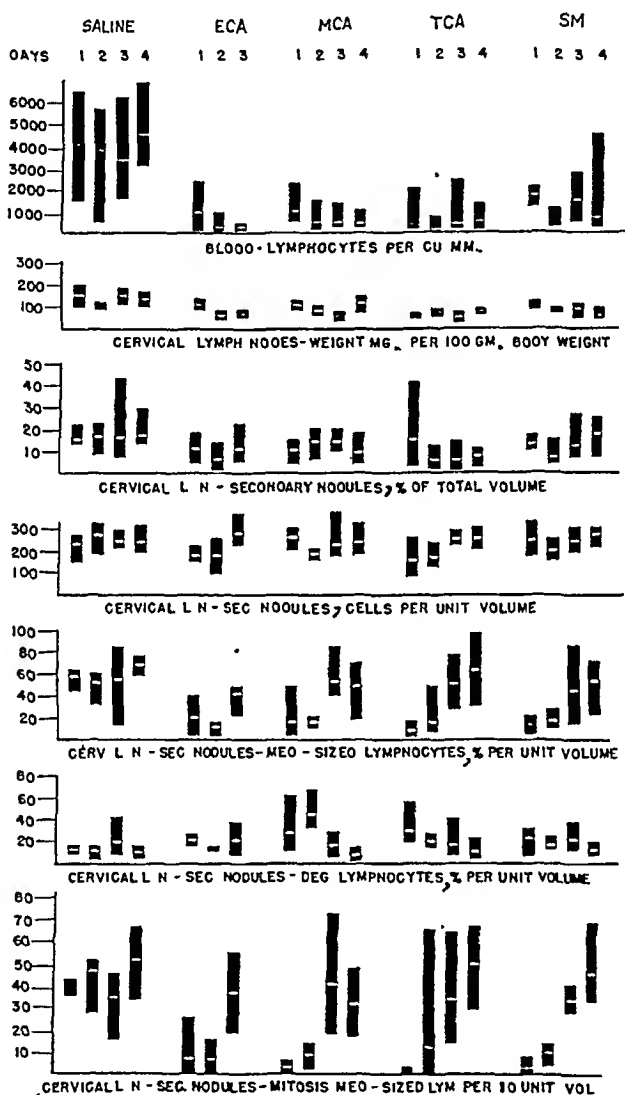


Fig. 4.—Block diagrams showing distributions of the lymphocytes of the circulating blood, the weight of the lymph nodes, the volume of the nodules and the numbers of cells of the nodules of the anterior cervical lymph nodes of rats one to four days after single injections of saline solution and vesicants. The mean, maximum and minimum values are indicated as in figure 1.

ume of the nodules remained lower than that of the control group throughout the remainder of the period, but in the ECA and SM groups the relative volumes reached the control level on the third day. Thus, only in the TCA group was the relative volume of the nodules markedly reduced. But it must be remembered



Fig. 5.—Photomicrographs of median vertical sections of anterior cervical lymph nodes; hematoxylin and eosin; sections 9 microns thick; $\times 16$. (a) Control one day after injection of saline solution. (b, c, d and e) One day, two days, three days and four days, respectively, after injection of tris (2-chloroethyl) amine.

that although the relative volumes of the nodules did not change much, the volumes of the whole nodes were significantly reduced by the action of the agents, so that actually there was significant reduction in the amount of lymphoid tissue in the nodules as a result of the action of the agents (figs. 5 and 6).

The secondary lymphoid nodule of the anterior cervical lymph node of the normal rat is a large ovoid structure which is roughly divided into two parts when viewed under the microscope in median vertical section (fig. 6a). There are a distal light zone, in which phagocytosis predominates and in which numerous macrophages of reticulum cell origin are seen filled with degenerated lymphocytes, and a proximal dark zone, in which medium-sized lymphocytes predominate, mitosis of these cells is prevalent and there are some macrophages. The secondary nodule is capped peripherally by a dense band of lymphocytes predominantly of the small variety.^{11a} The photograph of a median vertical section of a whole anterior cervical node (fig. 5a) and that of a median vertical section of a secondary nodule (fig. 6a) illustrate these relations. Qualitatively there was little change from this characteristic histologic pattern in the nodules of the control group throughout the period, but in all of the poisoned groups the distinction between the zones of the nodules was lost and there were no sharp lines of demarcation between the nodules and the surrounding cortex. The nodules were looser and less lymphoid and appeared degenerated. The general trend of change is shown in the photographs of sample nodules from the TCA group (fig. 6b to c).

In order to have histologic bases for contrasts of the conditions in the nodules, counts were made of the total number of cells and of the different types of cells per unit volume (90,000 cubic microns) of one nodule from one node of each rat used; and of the number of medium-sized lymphocytes in mitosis in one unit volume of each of ten nodules from one node of each rat used (fig. 4). These data were used with those of the weight of the nodes and the relative volume of the nodules to calculate the incidence of each of the different types of cells and of cells in mitosis per hundred grams of body weight. Since the calculated data convey a better idea of the trend of change, it was considered unnecessary to include all of the raw data in this paper. Data not included are on file in this laboratory and in the files of the Medical Division of the Chemical War Service.

Examination of the data in table 4A shows that in the control group there was no significant change in the average number of cells per hundred grams of body weight in the secondary nodules throughout the four day period. However, in all of the poisoned groups there were initial significant reductions in the average numbers of cells. The greatest decrease occurred in the TCA group. In the nitrogen mustard groups there were no further reductions in the number of cells throughout the period, but in the SM group there was further significant decrease on the second day followed by a rise on the third and fourth days.

There was no significant change in the number of large lymphocytes in the control group until the fourth day, at which time the number increased (table 4B). In the poisoned groups there was marked initial significant hypoplasia of large lymphocytes in the SM and TCA groups. Hypoplasia occurred in the ECA group on the second day and in the MCA group on the fourth day. In the SM group there was a trend toward increase during the remainder of the period, but in the TCA group there was no significant change after the second day.

The medium-sized lymphocytes did not change significantly in number in the controls throughout the period (table 4C). In the poisoned groups there was marked significant hypoplasia of these cells in all groups. It was greatest in the TCA group. The hypoplasia persisted in the ECA and TCA groups throughout the period. In the SM and MCA groups there were significant increases in the

11a. Kindred, J. E.: *Am. J. Anat.* 62:453, 1938.

numbers of medium-sized lymphocytes on the third day, and the increases continued through the fourth day.

The small lymphocytes were so variable in distribution that the only significant change occurred in the MCA group, where these cells were less numerous on

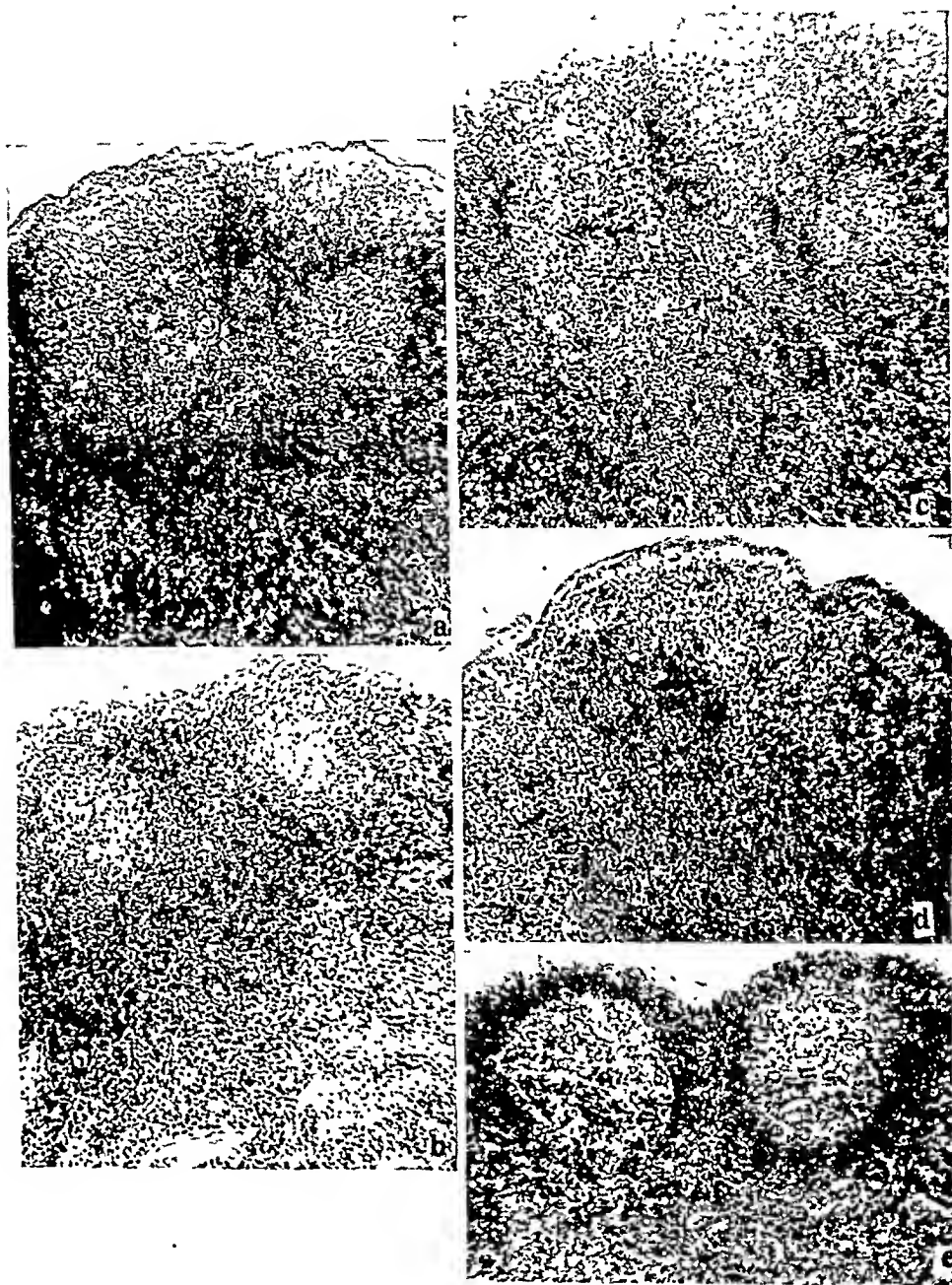


Fig. 6.—Photomicrographs of median vertical sections of secondary lymphoid nodules of anterior cervical lymph nodes, from sections shown in figure 5; $\times 58$. (a) Control one day after injection of saline solution. (b, c, d and e) One day, two days, three days and four days, respectively, after injection of tris (2-chloroethyl) amine.

the second day than on the first, although the number was not significantly different from that in the control group (table 4 D).

Degenerated lymphocytes fluctuated in number in the controls (table 4 E). Practically all of them were within macrophages. Among the poisoned groups, initially the SM and MCA groups had numbers that were not different from the

TABLE 4.—*Secondary Lymphoid Nodules of the Anterior Cervical Lymph Nodes of the Rats Used for the Study of the Thymus and Distributed in the Same Way in the Different Groups*

The counts recorded are the average numbers of cells of different types ($\times 10^4$) and of medium-sized lymphocytes in mitosis ($\times 10^3$) in secondary nodules per hundred grams of body weight. The data were calculated from the data in figure 4 and from unlisted data on large and small lymphocytes, reticulum cells and macrophages. Each average is accompanied by its standard error.

Days	Saline Solution	SM	EOA	MCA	TCA
A. Cells of All Types					
1	5,600 \pm 780	3,470 \pm 730*	2,550 \pm 250*	2,650 \pm 420	900 \pm 292*
2	4,000 \pm 560	1,200 \pm 360†	1,400 \pm 740	2,090 \pm 260	1,200 \pm 625
3	4,200 \pm 455	3,450 \pm 1,200	1,780 \pm 630	2,130 \pm 800	720 \pm 200
4	5,100 \pm 1,070	3,110 \pm 580	2,000 \pm 360	1,380 \pm 272
B. Large Lymphocytes					
1	227 \pm 58	30 \pm 11*	143 \pm 40	44 \pm 8*	24 \pm 16*
2	201 \pm 81	65 \pm 32	50 \pm 29†	154 \pm 46	37 \pm 23
3	144 \pm 25	90 \pm 25	78 \pm 30	150 \pm 47	47 \pm 20
4	300 \pm 50†	120 \pm 40	90 \pm 26	50 \pm 9
C. Medium-Sized Lymphocytes					
1	3,180 \pm 485	421 \pm 100*	618 \pm 160*	456 \pm 268*	94 \pm 41*
2	2,060 \pm 330	240 \pm 113	207 \pm 120	300 \pm 48	420 \pm 302
3	2,470 \pm 515	1,800 \pm 840†	500 \pm 160	1,300 \pm 500†	360 \pm 88
4	3,440 \pm 615	1,722 \pm 475	1,010 \pm 180	862 \pm 265
D. Small Lymphocytes					
1	1,031 \pm 380	1,860 \pm 735	880 \pm 221	1,200 \pm 261	377 \pm 146
2	1,166 \pm 200	620 \pm 148	812 \pm 420	600 \pm 181†	420 \pm 192
3	650 \pm 111	700 \pm 163	810 \pm 610	327 \pm 99†	183 \pm 90
4	466 \pm 190	710 \pm 310	690 \pm 170	260 \pm 60
E. Degenerated Lymphocytes					
1	734 \pm 110	720 \pm 170	2,690 \pm 125*	660 \pm 140	253 \pm 76*
2	390 \pm 49†	200 \pm 61†	186 \pm 100†	900 \pm 122	170 \pm 120
3	810 \pm 150†	700 \pm 210†	235 \pm 37	261 \pm 86†	90 \pm 32
4	638 \pm 268	400 \pm 102	100 \pm 22	135 \pm 31
F. Medium-Sized Lymphocytes in Mitosis					
1	910 \pm 102	30 \pm 13*	112 \pm 68*	22 \pm 13*	2 \pm 2*
2	740 \pm 130	48 \pm 16	36 \pm 45	88 \pm 27†	66 \pm 605
3	610 \pm 129	460 \pm 190†	216 \pm 625	360 \pm 170	99 \pm 43
4	1,070 \pm 192	480 \pm 220	280 \pm 85	270 \pm 66†
G. Reticulum Cells					
1	155 \pm 38	235 \pm 70	93 \pm 43	140 \pm 115	31 \pm 9*
2	110 \pm 61	54 \pm 10†	100 \pm 42	68 \pm 18	80 \pm 20
3	60 \pm 16	54 \pm 19	13 \pm 10	27 \pm 19	20 \pm 12†
4	70 \pm 19	48 \pm 16	50 \pm 12	25 \pm 7
H. Macrophages					
1	180 \pm 25	208 \pm 39	175 \pm 112	154 \pm 49	116 \pm 44
2	80 \pm 24*	43 \pm 13†	25 \pm 8†	70 \pm 40	24 \pm 8
3	132 \pm 23	120 \pm 32	70 \pm 26	70 \pm 22	21 \pm 5
4	166 \pm 42	106 \pm 25	50 \pm 10	50 \pm 7†

* The number is significantly different from the mean of the first day controls (saline solution).

† The number is significantly different from the mean of the preceding day.

number in the control group, but in the ECA group there was an initial increase and in the TCA group a decrease. On the second day the numbers of all but the MCA group decreased. On the third day there occurred increase in the SM group no change in the ECA and TCA groups and decrease in the MCA group.

There were no significant changes on the fourth day in the surviving rats. Calculations showed that the number of lymphocytes lost could not be accounted for by the degenerated cells; hence some of this loss must have been due to rapid emigration of cells or to the macrophages' rapid digestion of injured cells.

There was no significant change in the number of medium-sized lymphocytes in mitosis in the control group throughout the period (table 4 F). In all the poisoned groups, however, there was marked significant decrease of the numbers of lymphocytes in mitosis. The decrease was greatest in the TCA group. Significant recovery of mitotic activity began in the MCA group on the second day. On the third day there was significant increase in the SM group and in some rats of the ECA and TCA groups. It did not reach significant proportions in the TCA group until the fourth day. Because there was greater proportional decrease initially in the numbers of medium-sized lymphocytes in mitosis than in the numbers of medium-sized lymphocytes, it is concluded that mitotic activity was inhibited for at least two days in all but the MCA group.

The number of unmodified reticulum cells did not change in the control group throughout the period (table 4 G). In the poisoned groups, the number decreased initially only in the TCA group. It fluctuated in this group after the first day, but reticulum cells were always less numerous than in the controls. In the SM group there was a marked decrease on the second day, and in the ECA and MCA groups on the third day. Unlike those noted in the thymus, these scattered decreases did not occur because these cells became macrophages, for there were no initial increases in the numbers of macrophages such as were seen in the thymus (table 4 H). The macrophages tended to decrease in the poisoned groups, but the individual variations were so great that one can conclude only that there is a greater trend toward decrease than toward stability. This trend also occurred in the thymus. A contrast of the data concerning the macrophages of the lymph nodes and of the thymus indicates that there is a physiologic difference between the macrophages. In the lymphoid nodules the macrophages are always present and are continually selecting, ingesting and digesting injured lymphocytes, while in the thymus phagocytosis is apparently a secondary function and is brought about by the production of phagocytosis-stimulating substances around unmodified reticulum cells. This substance is apparently found around degenerating cells, a fibrin-like deposit which makes the surface of the injured cell sticky. These sticky cells when coming into contact with a reticulum cell may stimulate it to become a phagocyte. Another difference between the macrophages of the thymus and those of the lymphoid nodules was the accumulation of degenerated cells in the macrophages of the thymus and the little increase of the number normally seen in the macrophages of the lymphoid nodules. The absence of accumulations of injured lymphocytes in the macrophages of the lymphoid nodules is thought to be caused by their rapid ingestion and digestion. Such a view is based on the belief that these macrophages normally contain an active cytolytic enzyme. Many of the macrophages in both the thymus and the lymphoid nodules perished as a result of the burden imposed on them by the local destruction of cells. This seemed to be particularly true on the second day after injection. From examination of the relation of the macrophages and the degenerated cells it is believed that in both the thymus and the lymph node the disintegration of macrophages and the reduction in their number were not the result of direct toxic action of the agents but were due to exhaustion following excessive phagocytosis.

As a result of the destruction of the larger lymphocytes and the disintegration of some of the macrophages, the lymphoid nodules became very loose, and there were wide edematous spaces between the cells (fig. 6 b). However, there was no

complete necrosis, and after the wave of destruction had passed, the nodules again became more compact and regeneration began (figs. 6*d* and 8). Regeneration of the larger lymphocytes of the nodules could be from two sources: small lymphocytes which had been temporarily inhibited from growing by the action of the agents and reticulum cells which had been uninjured by the agents. There appears to be more evidence from the distributions of the small lymphocytes in the nodules on the third and fourth days for the first view.

In brief, it appears that the agents caused destruction of the larger lymphocytes in the secondary nodules of the lymph nodes, inhibition of mitosis in these cells and inhibition of growth of smaller into larger forms. The macrophages rapidly ingested and digested the injured cells, and many perished as a result of this activity. The nodules became small, edematous and loose as a result of these changes. The toxic effects of the agents lasted for about two days and were followed by the beginning of regeneration of medium-sized lymphocytes and resumption of mitosis. The changes in the nodules of the lymph nodes paralleled the changes in the cortex of the thymus and the changes in the blood picture. While the lymphopenia of the blood was at its height, the destruction of lymphocytes and the inhibition of mitosis were greatest. These changes in the thymus and the lymphoid nodules are thought to be directly related to the lymphopenia of the blood. That there was not complete lymphopenia during this period of inhibition of lymphopoietic activity is thought to have been due to the fact that uninjured lymphocytes migrated from these organs. Migration of uninjured lymphocytes is believed to be the chief reason for the shrinkage of the thymus and the lymph nodes.

The medullas of the lymph nodes shrank in proportion to the shrinkage of the whole organs in the poisoned groups (fig. 5). These decreases probably occurred as lymphocytes migrated from the medullary cords. The plasma cells which form the bulk of the population of the medullary cords were apparently uninjured by the agents.

THE SPLEEN

In the normal rat the spleen plays a minor role in the hemopoietic system. The active lymphocytopoietic centers (malpighian nodules) produce relatively small numbers of lymphocytes compared with the thymus and the cervical lymph nodes. Granulocytes and erythrocytes and blood platelets are also produced in the spleen, but the numbers of these cells contributed to the circulating blood are small.¹ The spleen was studied in detail, however, by the same methods which were used for the study of the thymus and the lymph nodes, in order to ascertain whether the tissues had been affected in the same manner by the vesicants as had those of the thymus and the lymph nodes. Since the spleen plays such a minor role in hemopoiesis, it was decided not to include the detailed tables which were made from the quantitative study. It was felt, however, that a brief résumé should be given concerning the histologic conditions observed in the spleens of the poisoned rats. The most important trends of the changes observed in the spleen as a whole and in the splenic nodules are shown in figure 7.

The average weight of the spleen decreased significantly in the control group during the first two days after injection, but on the third and fourth days showed a trend toward increase (fig. 7). In all of the poisoned groups the weight of the spleen decreased significantly one day after injection and remained below the control level throughout the period. The relative volume of the lymphoid tissue was initially reduced in the poisoned groups but regenerated by the fourth day in all but the TCA group (fig. 7). The decrease of the weight of the spleen was

probably caused not by the decrease of the lymphoid tissue but by the contraction of the sinuses, which occurred in all of the poisoned groups and which was frequently accompanied by fibrosis. Red blood cells which normally occupy the sinuses and the intersinal spaces of the spleen were few in the poisoned rats, and the reticulum cells were contracted and formed dense masses. The changes in the size of the spleen are shown in the photographs of sections taken at the level of the hilus (fig. 8).

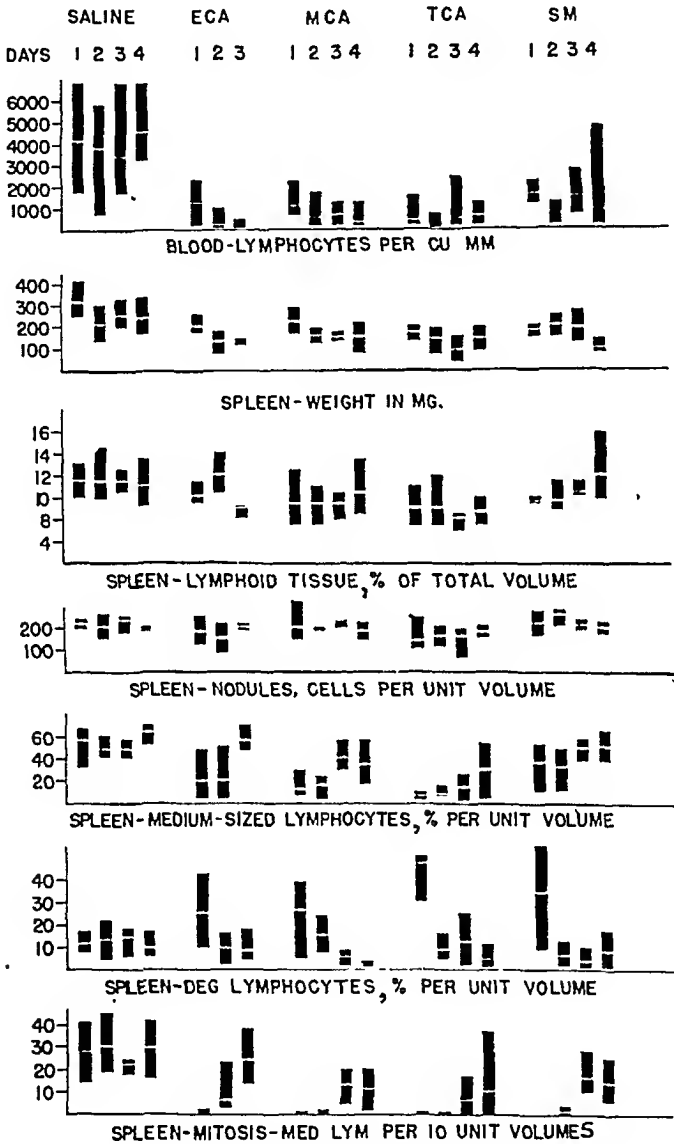


Fig. 7.—Block diagrams showing distributions of lymphocytes of the circulating blood, the weight of the spleen, the volume of the lymphoid tissue of the spleen and the cell counts of secondary lymphoid nodules of the spleen of rats one to four days after injection of saline solution and vesicants. The mean, maximum and minimum values are indicated as in figure 1.

As in the thymic cortex and in the cortex of the lymph node outside of the nodules, the splenic lymphoid cords of the poisoned rats showed initial hypoplasia of lymphocytes, which resulted from local destruction of lymphocytes and loss due to migration. The degenerated lymphocytes were ingested and digested by macro-

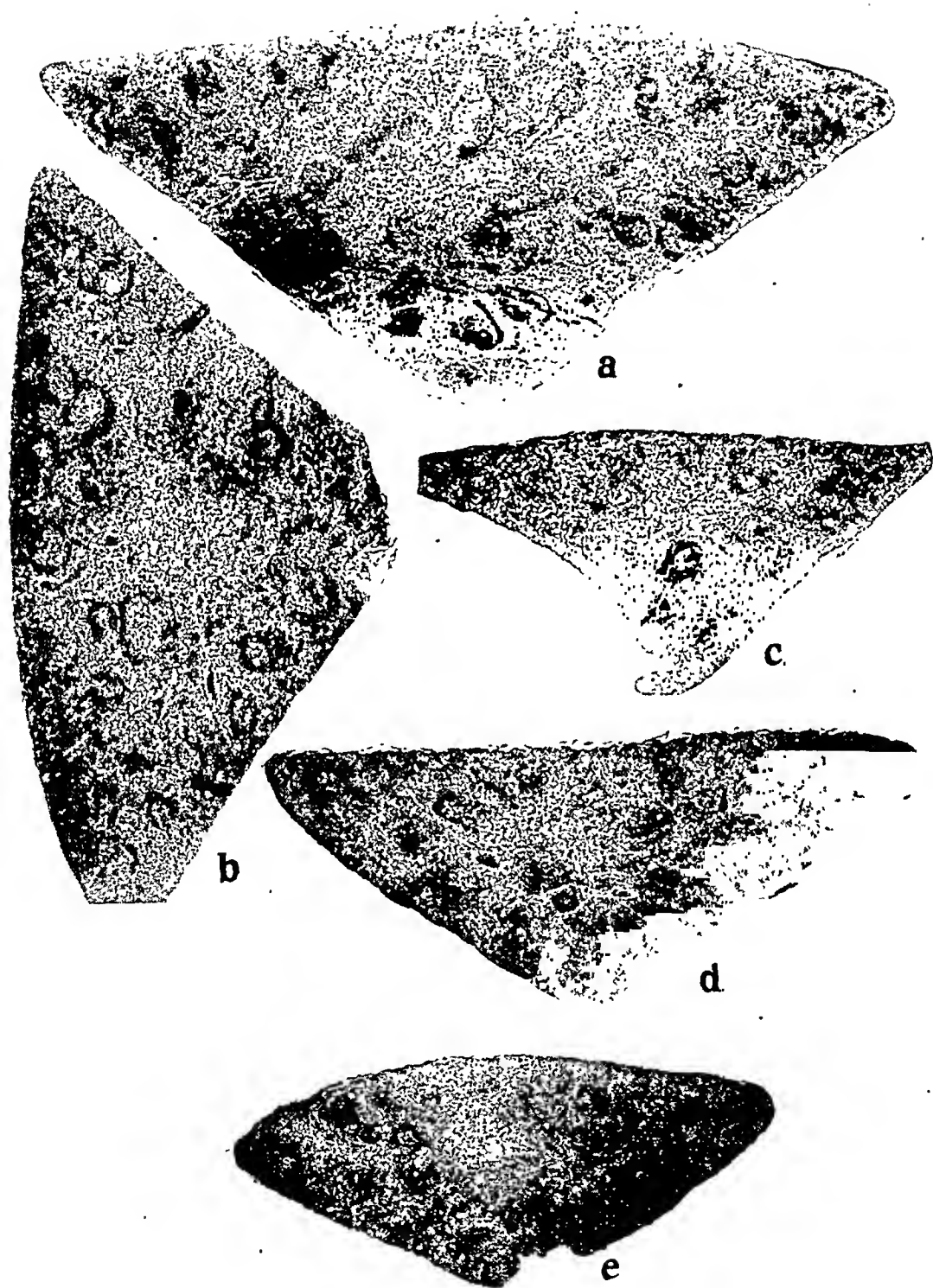


Fig. 8.—Photomicrographs of transverse sections of the spleen taken at the level of the hilus; hematoxylin and eosin; sections 7 microns thick; $\times 16$. (a) Control one day after injection of saline solution. (b, c, d and e) One day, two days, three days and four days, respectively, after injection of tris (2-chloroethyl) amine.

phages of reticulum cell origin. Normally, in this part of the spleen there is little destruction, and few active macrophages are present. As a result of the loss of lymphocytes, the stroma became loose, and reticulum cells and blood capillaries were conspicuous (fig. 9 *b* to *e*). The cords became narrow and were often invaded by fibroblasts. The small degree of mitosis normally present was inhibited. Regeneration of large and medium-sized lymphocytes began on the

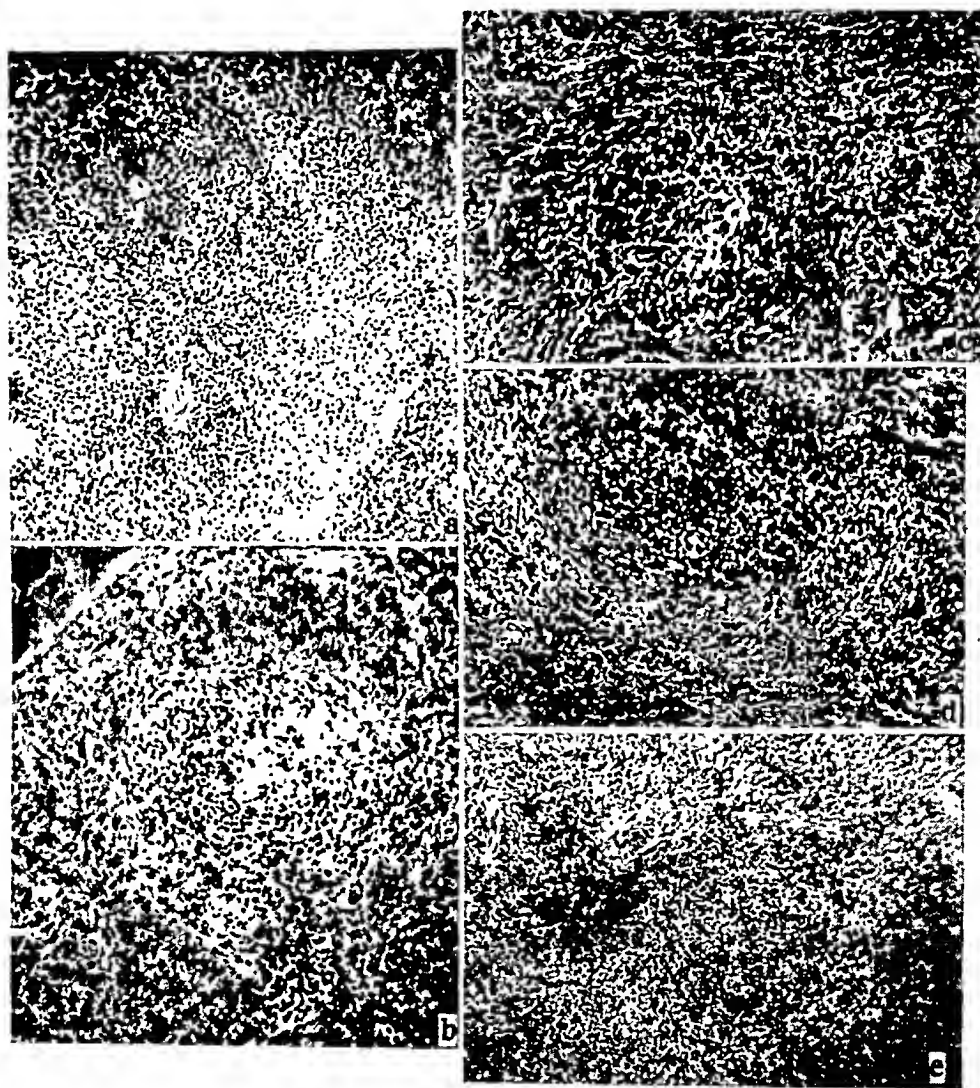


Fig. 9.—Photomicrographs of median vertical sections of secondary lymphoid nodules and adjacent lymphoid cords, from sections shown in figure 8; $\times 54$. (*a*) Control one day after injection of saline solution. (*b*, *c*, *d* and *e*) One day, two days, three days and four days, respectively, after injection of tris (2-chloroethyl) amine.

third day, but the number of small lymphocytes continued to decrease throughout the period. Mitotic activity was resumed in all poisoned groups by the third day but remained significantly lower than in the control group throughout the period. The TCA group showed the slowest regeneration. The reticulum cells were

reduced in number, possibly by the edema which separated them as the lymphocytes were destroyed or migrated, but probably the decrease was caused by their being transformed into macrophages. Throughout the later part of the period there was no consistency in the distribution of reticulum cells and macrophages. It is possible that this inconsistency was due to the degeneration of the macrophages following their novel phagocytic activity. On the whole there is marked similarity between the changes occurring in the splenic cords and those in the thymic cortex and the cortex of the lymph node.

The splenic nodules (malpighian nodules) are normally sites of a minor degree of destruction and proliferation of lymphocytes. They contribute few lymphocytes to the general circulation.¹ There are no light and dark zones in these nodules such as are present in the nodules of the lymph nodes, and the nodules are surrounded by a dense zone of unmodified reticulum cells (fig. 9 *a*). These histologic conditions were maintained in the control rats. In the poisoned rats there was hypoplasia of lymphocytes in the nodules and increase of the number of degenerated lymphocytes in the macrophages (fig. 7). The nodules became loose, and the reticulum-macrophage stroma became prominent. Photographs of samples from the TCA group illustrate these initial changes (fig. 9 *b*). The peripheral zone of reticulum cells loosened and was infiltrated by red blood cells from the neighboring sinuses. Mitosis of the medium-sized lymphocytes was inhibited (fig. 7). Regeneration of the nodules, characterized by increase in the number of medium-sized lymphocytes and resumption of mitosis, began on the third day (fig. 7). At the same time degenerated lymphocytes and macrophages decreased in numbers, and the surrounding reticular stroma became more compact (fig. 9 *c* to *e*). The sequence of destruction and regeneration paralleled, both in general character and in time, that which occurred in the nodules of the lymph nodes except in one respect. This particular difference was in the initial increase of the number of degenerated lymphocytes contained within the macrophages of the splenic nodules, whereas it will be recalled that degenerated cells decreased or did not change significantly in number in the macrophages of the lymphoid nodules of the lymph nodes. Such a difference suggests that the cytolytic enzymes of the macrophages of the splenic nodules are not as active as those of the macrophages of the lymph nodes.

The granulocytopoietic and erythrocytopoietic areas did not change in volume in the controls throughout the period. But in the poisoned rats there were reductions in the numbers and in the mitotic activity of the myelocytes and the erythroblasts for two days after the injection. The reduction in the numbers of cells appeared to be due to loss by migration since there was no evidence of local destruction of cells such as occurred in the lymphoid parts of the spleen. The megakaryocytes in these areas were unaffected by the agents.

The venous sinuses were collapsed or contracted in the poisoned rats and were mostly devoid of red blood corpuscles, which characterize these regions in the controls. In most of the rats the spleen had large areas of fibrosis, which originated from the hilus. In some rats the whole spleen was infiltrated with fibroblasts. Despite these changes there was no evidence of localized necrosis. There was no evidence of increased destruction of red blood corpuscles.

In brief, the lymphoid tissue of the spleen experienced the same kind of degeneration which characterized the lymphoid tissue of the cortices of the thymus and the lymph nodes; in addition its function as an accessory myeloid and erythroid center was temporarily depressed. Likewise, its function as a storage reservoir for red blood corpuscles was reduced.

COMMENT ON THE LYMPHOID ORGANS

In table 5 are presented in brief form contrasts between the numbers of lymphocytes of the blood and the amounts of lymphoid tissue observed in the thymic cortex, in the secondary nodules of the cervical lymph nodes and in the spleen in the poisoned rats on the first and fourth days after injection. These data are given in terms of percentage of the average value of each item as obtained for the control rats at the end of the first day after injection. The purpose of the table is to contrast briefly the degrees of reduction of the lymphoid tissue of the several organs caused by the vesicants.

It will be seen in table 5 that sulfur mustard produced the least initial effect on the number of lymphocytes of the blood and on the

TABLE 5.—Percentages of First Day Control Values to Which Lymphocytes of Blood and Lymphoid Tissues of Thymic Cortex, Secondary Nodules of Cervical Lymph Nodes and Lymphoid Tissue of Spleen Had Been Reduced on First and Fourth Days, Respectively, After Injection of Vesicants*

Cells or Organ	Day	Saline Solution	SM	ECA†	MCA	TOA
Lymphocytes.....	1	100 ± 7.2	42 ± 5.1	27 ± 10.2	27 ± 3.9	11 ± 2.0
	4	111 ± 16.0	21 ± 14.0	8 ± 1.5	11 ± 1.0	13 ± 2.8
Thymus.....	1	100 ± 3.5	50 ± 10.5	60 ± 3.0	55 ± 6.1	50 ± 10.5
	4	56 ± 6.5	16 ± 1.6	19 ± 1.9	23 ± 2.5	16 ± 1.6
Lymph nodes.....	1	100 ± 10.0	63 ± 9.2	50 ± 14.0	46 ± 8.8	21 ± 6.2
	4	87 ± 13.0	52 ± 12.5	25 ± 8.8	37 ± 6.2	23 ± 3.7
Spleen.....	1	100 ± 15.0	80 ± 14.5	62 ± 6.0	64 ± 11.0	44 ± 4.9
	4	80 ± 14.5	54 ± 25.0	35 ± 3.1	62 ± 7.0	39 ± 18.0

* Each value is followed by its standard error.

† The values in the ECA group are given for the third day, since there were no survivors beyond this day.

amounts of lymphoid tissue of all organs except the thymus; tris (2-chloroethyl) amine had the greatest effect, while ethyl-bis (2-chloroethyl) amine and methyl-bis (2-chloroethyl) amine produced almost identical effects intermediate between those of sulfur mustard and tris (2-chloroethyl) amine. The lymphoid tissue of the thymic cortex was affected initially less than that of the spleen in all except the ECA group. In all but the SM group, however, the secondary lymphoid nodules of the lymph nodes were reduced by a greater degree than either the lymphoid tissue of the thymic cortex or that of the spleen. By the fourth day the lymphoid tissue of the thymic cortex had been reduced by a greater degree in all groups than that of either the spleen or the lymph nodes. The greatest relative reduction occurred in the ECA group. The only significant further reduction of the lymphoid tissue of the lymph nodes and the spleen occurred in the spleen in the ECA group.

In relating these changes to the changes in the number of lymphocytes of the circulating blood, it appears that the chief factor responsible for the drastic reduction of the number of lymphocytes which occurred was the decrease in the amount of lymphoid tissue of the thymic cortex and the depression of its lymphocytopoietic activity. The acute changes in the thymus may have been shared by the lymph nodes and the spleen, but the lymphocyte production of these organs is normally only a fraction of that of the thymus. A rough calculation based on the data concerning the incidence of cells in mitosis in the thymic cortex, the secondary nodules of the cervical lymph nodes and the spleen shows why this occurs. From these data it has been estimated that if it takes one hour for the completion of a mitotic cycle, the thymic cortex of the control rat could produce per hour about 10 per cent of the number of lymphocytes of the blood per hundred grams of body weight, whereas the cervical lymph nodes could produce about 3.0 per cent and the spleen about 0.4 per cent. At the end of the first day after the injection of the vesicants the number of lymphocytes produced by the thymic cortex, it has been estimated, would have dropped to such a degree that only about 0.4 per cent of the normal complement of lymphocytes would be produced per hour in the SM group, 0.7 per cent in the ECA group, 2.2 per cent in the MCA group and 0.2 per cent in the TCA group. In the secondary nodules of the cervical lymph nodes the estimated production dropped to about 0.09 per cent in the SM group, to 0.35 per cent in the ECA group, to 0.06 per cent in the MCA group and to 0.006 per cent in the TCA group. And in the secondary nodules of the spleen the drop was to 0 in the SM group, to 0.005 per cent in the ECA and MCA groups and to 0.002 per cent in the TCA group. Thus it is evident that the lymphopenia of the poisoned rats is largely due to the decrease of the number of lymphocytes produced by the thymic cortex and only to a lesser degree to the decrease of the numbers produced by the lymphoid tissue of the cervical lymph nodes and the spleen, even though the lymphoid tissue of the thymus did not decrease relatively as much as did that of the cervical lymph nodes and that of the spleen. Furthermore, the decrease of the numbers of lymphocytes of the circulating blood of the poisoned rats was of the same degree as that observed in rats from which all of the lymphoid tissue (93 per cent) except that of the alimentary tract¹² had been removed. It has been shown, moreover, that the spleen plays a minor role in relation to the lymphocytes of the blood of the rat, since there is no change in the count of the white cells of the peripheral blood of rats¹³ after splenectomy.

The injection of the adrenocorticotrophic hormone of the pituitary gland, which causes acute hypoplasia of the thymus, the spleen and the

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13. Higgins, G. M., and Stasney, J.: *Folia haemat.* **56**:189, 1936.

lymph nodes, is followed by an acute numerical decrease of the lymphocytes of the circulating blood in the mouse¹⁴ and in the rat and the dog.¹⁵ From the original investigations of Heineke¹⁶ and many later investigations¹⁷ it has been shown that in small animals exposed to roentgen rays acute degenerative changes develop in the lymphocytes of the thymus, the lymph nodes and the spleen, accompanied by lymphopenia of the blood. Thus the belief that the lymphopenia of the rats poisoned by injected vesicants is caused by degenerative changes of the lymphoid organs, particularly of the thymus, receives support from others' investigations of the relation between acute degeneration of the lymphoid organs and lymphopenia of the blood.

The same acute degenerative effects are produced in the thymus and the lymph nodes by agents which cause the "alarm reaction" in rats,¹⁸ and they result in transient lymphopenia of the blood.¹⁹ The acute degenerative changes caused by compounds which cause the "alarm reaction" are prevented by adrenalectomy,²⁰ as are those caused by the adrenocorticotrophic hormone.²¹ Likewise, the lymphopenia which accompanies these changes does not occur in adrenalectomized animals.²² According to these investigators, the cortex of the adrenal gland is the mediator of the substance which produces the degenerative effect. In animals treated with compounds causing the "alarm reaction" the adrenal cortex hypertrophies and the lipid content decreases.²⁰ In animals given injections of adrenocorticotrophic hormone there is depletion of the sudanophilic material and of the cholesterol of the adrenal cortex.²³ In dogs irradiated with roentgen rays there is congestion of the cortex,²⁴ and in rabbits also.²⁵ No change was found in the adrenal glands of mice which

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15. Reinhardt, W. O.; Aron, H., and Li, C. H.: *Proc. Soc. Exper. Biol. & Med.* **57**:19, 1944.

16. Heineke, H.: *Mitt. a. d. Grenzgeb. d. Med. u. Chir.* **14**:21, 1905.

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19. Harlow, C. M., and Selye, H.: *Proc. Soc. Exper. Biol. & Med.* **36**:141, 1937.

20. Leblond, C. P., and Segal, G.: *Compt. rend. Soc. de biol.* **129**:838, 1938. Selye.¹⁸

21. (a) Simpson, M. E.; Li, C. H.; Reinhardt, W. O., and Evans, H. E.: *Proc. Soc. Exper. Biol. & Med.* **54**:135, 1943. (b) Dougherty, T. F., and White, A.: *Am. J. Anat.* **77**:81, 1945.

22. Dougherty and White.¹⁴ Reinhardt and others.¹⁵

23. Dougherty, T. F., and White, A.: *Endocrinology* **35**:1, 1944. Sayers, G.; White, A., and Long, C. H. N.: *Proc. Soc. Exper. Biol. & Med.* **52**:199, 1943.

24. Shouse, S. S.; Warren, S. L., and Whipple, G. H.: *J. M. Research* **53**:421, 1931.

25. Harvey, W. G.: *J. Path. & Bact.* **12**:548, 1908.

had been treated with roentgen rays or neutrons,²⁶ but after irradiation of rats the cells of the adrenal cortex hypertrophied and lost fat.²⁷ In rabbits poisoned by injected sulfur mustard there was acute congestion of the adrenal glands,^{4b} and in rabbits in which the skin had been contaminated with sulfur mustard there was decrease of cortical lipid materials.²⁸ In rats receiving injections of methyl-bis (2-chloroethyl) amine there were 60 per cent increase of the weight of the adrenal glands, hypertrophy of the cortex and depletion of the lipid.²⁹ In the rats used in the present experiments the adrenal glands were not hypertrophied, but there was increase in the size of the cells of the spongy zone of the zona fasciculata and decrease in the number of fat vacuoles of the adrenal cortex. Ludewig and Chanutin³⁰ observed increase of weight with decrease of cholesterol in the adrenal glands of a larger group of rats from this same colony which had received injections of vesicants. Thus it appears that the lymphoid changes accompanying injections of compounds causing the "alarm reaction," roentgen irradiation and other treatments and interpreted as having been caused by changes occurring in the metabolism of the cells of the adrenal cortex are paralleled by similar morphologic and physiologic changes in the adrenal glands of rats poisoned with vesicants. As pointed out, the degenerative changes following the administration of compounds causing the "alarm reaction" and that of adrenocorticotrophic hormone are prevented by adrenalectomy. However, the degenerative changes following roentgen irradiation of rats are not prevented by adrenalectomy if the thymus is not shielded,²⁷ and adrenalectomized rats poisoned by injected methyl-bis (2-chloroethyl) amine show the same degenerative changes as do the nonadrenalectomized rats, although the effect may not be quite as great.³¹ This decrease of effect was also noted by Leblond and Segal²⁰ in adrenalectomized rats which had been exposed to roentgen rays.

In the present investigation, adrenalectomized rats received injections of a variety of nonlethal doses of tris (2-chloroethyl) amine. The lymphoid organs showed degenerative changes of the same type and degree as those observed in the lymphoid organs of nonadrenalectomized rats, and the damage was proportional to the amount injected. Hence it is concluded that the vesicants produce some of their intoxicating effects on the lymphoid organs directly, although they in part may be

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27. Segal, G., and Leblond, C. P.: *Compt. rend. Soc. de biol.* **129**:279, 1938.

28. Cameron, G. R., and Short, R. H. D.: Unpublished data, 1942.

29. Karnofsky, D. A.; Graef, I., and Addis, E.: Unpublished data, 1942-1943.

30. Ludewig, S., and Chanutin, A.: *Endocrinology* **38**:376, 1946.

31. Karnofsky, D. A.; Graef, I., and Smith, H. W.: *Federation Proc.* **5**:224, 1946.

mediated by the adrenal cortex. This conclusion is in agreement with that of Karnofsky and associates.³¹

At this point it seems pertinent to inquire into the mechanism by which the mustards act on the lymphoid cells. In a series of *in vitro* observations of lymphocytes of the thymus which were suspended in plasma to which tris (2-chloroethyl) amine had been added in a proportion equivalent to that obtaining in the solution injected, it was found that the nuclei of the cells were injured within two hours after the beginning of the exposure. The nucleus first became opaque, and then the chromatin broke up into small vesicles which flowed together. Subsequently the nuclear membrane seemed to dissolve, and the chromatin globules were scattered through the cytoplasm. In fixed materials this same sequence of events was observed, and in sections treated with Feulgen's reagent the chromatin particles gave a positive reaction for desoxyribonucleic acid, in which the thymus is particularly rich.³² It is thought that during the active life of the cell the desoxyribonucleic acid is being continually built up and broken down,³² a metabolic condition which has been detected by the use of ultraviolet rays³² and radioactive phosphorus.³³ The desoxyribonucleic acid is particularly unstable in cells which are actively dividing,³² such as the medium-sized lymphocytes of the thymus and of the centers of the nodules of the lymph nodes and the spleen. The vesicants stop this activity. The question may be asked whether the agents damage the protein, the desoxyribonucleic acid or the enzyme system responsible for the maintenance of nuclear and cytoplasmic function. If the Feulgen reaction is taken to be a positive indication of the presence of desoxyribonucleic acid, the vesicants do not destroy this substance, as it still can be seen in the nuclei of degenerated cells even after they have been ingested by macrophages. Since the cells are no longer actively dividing, it is possible that the vesicants may depolymerize the desoxyribonucleic acid and deactivate it in much the same way as sulfur mustard has been reported to deactivate certain viruses.³⁴ Radium causes similar nuclear degeneration of cells *in vitro*³⁵; and *in vitro* cells of the thymus of the rabbit undergo degeneration when exposed to roentgen rays, and the degeneration is proportional to the dose administered.³⁶ As yet, however, there has been no demonstration of all of the chemical changes which could account for

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33. Andreasen, E., and Ottensen, J.: *Acta path. et microbiol. Scandinav.*, 1944, supp. 54, p. 25.

34. Tenbroeck, C., and Herriott, R. M.: *Proc. Soc. Exper. Biol. & Med.* 62:271, 1946.

35. Cinti, R. G.: *Acta radiol.* 10:320, 1929.

36. Schrek, R.: *Radiology* 46:395, 1946.

the morphologic changes which have been observed in the nuclear chromatin of cells injured by physical and chemical agents. For discussions of the problems relating to these changes the reader is referred to Gilman and Phillips⁵ and Greenstein.³²

As has been stated, there is lymphopenia of the circulating blood at the same time that degenerative changes are going on in the lymphoid organs of the poisoned rats. The question arises as to the fate of the lymphocytes of the circulating blood. With the inhibition of mitosis, lymphocytes are not entering the blood stream in the numbers which are normally expected, and there is no evidence from the blood smears that they are degenerating in the blood stream. The fact that the uninjured lymphocytes are drained from the lymphoid organs is believed to account for the continued shrinkage of the lymphoid tissue of the several organs studied, and this drainage can prevent complete lymphopenia, but it does not account for the original loss of lymphocytes. Bunting and Huston³⁷ have suggested in regard to normal rabbits that the lymphocytes are lost from the body through the intestinal epithelium. Jordan³⁸ has suggested that the lymphocytes which are in excess of needs of the circulating blood are filtered off into the bone marrow, where they act as ancestors of the erythroblasts. Recently Farr³⁹ has shown that lymphocytes marked with acroflavine when introduced into the circulation of rabbits remain in the circulation for sixty to seventy-two hours and then disappear. Marked lymphocytes were found in the marrow and to a lesser extent in the lymphoid tissues, but no statement was made about their relation to the intestinal mucosa. In the rats which received injections of tris (2-chloroethyl) amine there were no accumulations of lymphocytes in such tissues as the lungs, the stomach, the trachea, the liver, the nervous system or the bone marrow, but large numbers of degenerated and uninjured lymphocytes were found in the lamina propria and in the lumen of the small intestine in regions where the epithelium of the villi had been sloughed off or where the epithelial cells had been injured. In the normal rat, it has been calculated, there could be $125,000,000 \pm 32,000,000$ small lymphocytes in the intestinal epithelium per hundred grams of body weight.¹ This number amounts to about three times the number of lymphocytes estimated to be present in the circulating blood at any one time. It was thought that in the normal rat these lymphocytes could not pass through the epithelium because of the intercellular cement present between the termini of the epithelial cells.⁴⁰ Recently Andrew and Andrew⁴¹ have suggested that the lymphocytes

37. Bunting, C. H., and Huston, J.: *J. Exper. Med.* **33**:593, 1921.

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escape by this route in the normal mouse; in the rat such a fate has been suggested.⁴² Since in the normal rat great numbers of lymphocytes do occur in the intestinal mucosa, and since this mucosa is injured by the vesicants, it is believed that the initial lymphopenia results because lymphocytes are lost through this region. Eosinophilic granulocytes also seemed to be leaving the body through this region. Hence it is suggested that the decrease in their incidence noted in the poisoned rats may be partially explained by their leaving through this route.

In animals treated with compounds causing the "alarm reaction" enteritis occurs,¹⁸ and since in animals subjected to roentgen irradiation there is distinct injury of the intestinal mucosa⁴³ and the other changes described in the vesicant-injured animals are characteristic of animals irradiated with roentgen rays, including lymphopenia, it is suggested from the nature of the syndrome that the lymphocytes are lost from the body through the intestinal mucosa. Other investigators have found the intestinal mucosa to be damaged after intoxications due to sulfur mustard,⁴⁴ ethyl-bis (2-chloroethyl) amine,^{44c} methyl-bis (2-chloroethyl) amine⁴⁵ and tris (2-chloroethyl) amine,⁴⁶ respectively. In man the lymphopenia following injection of methyl-bis (2-chloroethyl) amine and tris (2-chloroethyl) amine is accompanied by nausea.⁴⁷

THE FEMORAL MARROW

The marrow of the femur of the normal rat is dense; there is little fat, and few sinusoids are discernible. The marrow of the control group was like this (fig. 11 *a*). In the control group there was no change in the average number of cells per unit volume (50,000 cubic microns) throughout the four day period (fig. 10 and table 6 *A*). In the poisoned groups the marrows were slightly hypoplastic and more hyperemic than the marrow of the control group at the end of the first day (fig. 11 *b*). The greatest hypoplastic changes occurred in the TCA and SM groups (fig. 10 and table 6 *A*). Changes of this type were less marked in the ECA and MCA groups, although there was individual variation. The numbers of cells were significantly reduced in the SM and TCA groups. On the second day there was marked significant cellular hypoplasia with concomitant hyperemia in all the poisoned groups (fig. 11 *c*), and the average numbers of cells per unit volume were significantly less than the control average. In sections of the marrow

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43. Warren, S. L., and Whipple, G. H.: (*a*) *J. Exper. Med.* **35**:187 and (*b*) 202, 1922; (*c*) **38**:741, 1923. Shouse and others.²⁴ Lawrence and Tennant.²⁶

44. (*a*) Courtice, F. C., and Cameron, G. R.: Unpublished data, 1942. (*b*) Cameron, G. R., and Short, R. H. D.: Unpublished data, 1942. (*c*) Graef, I.; Karnofsky, D. A.; Jager, J. B., and Smith, H. W.: *Federation Proc.* **5**:221, 1946; unpublished data, 1945.

45. (*a*) Crawford, B., and Smith, H. W.: Unpublished data, 1944. (*b*) Graef and others.^{44c}

46. (*a*) Short, R. H. D.: Unpublished data, 1943. (*b*) Hanck, C. R.: Unpublished data, 1944.

47. Goodman and others.^{6b} Jacobson and others.^{6c}

the cells were scattered between the enlarged sinusoids, and there were large areas in which only cell remnants, fibrin and stroma could be seen. These changes produced a reduction in the amount of active marrow (fig. 10). Red blood corpuscles occupied many of these spaces, and it appeared as though these spaces

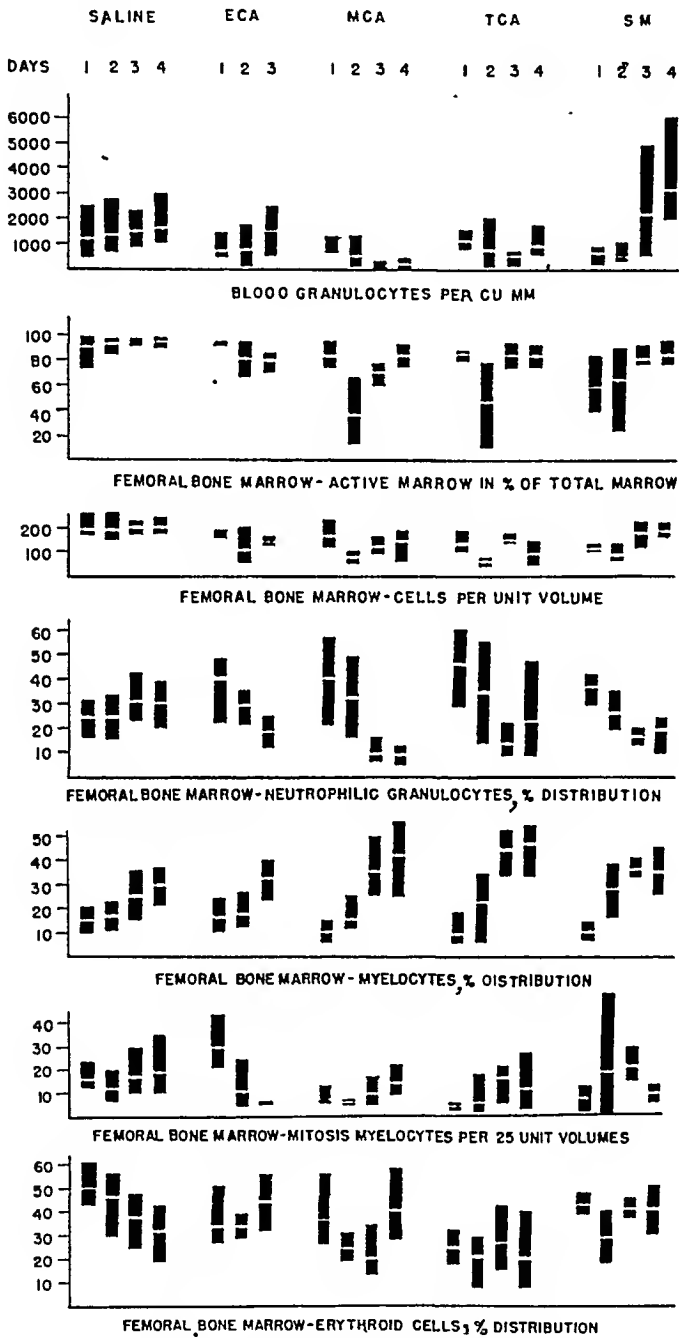


Fig. 10.—Block diagrams showing distributions of the neutrophilic granulocytes of the circulating blood, the percentage volume of active marrow and distributions of cells of the femoral marrow of rats one to four days after injection of saline solution and vesicants. The mean, maximum and minimum values are indicated as in figure 1.

were continuous with the sinusoids. The cells for the most part were uninjured, but there were some cells with pyknotic nuclei. In some marrows there were masses of fibrin, a sure sign of cellular injury. The reticulum cells and the endothelial cells were prominent, and the characteristic grouping of erythroblasts into scattered islands was absent. The osteoblasts on the endosteum were unchanged. The marrow when removed to make smears had a watery consistency. Conditions more or less like this persisted in the ECA, MCA and TCA groups throughout the period, but there were significant increases in the cell populations toward the end of the period, although they still remained below the normal level. In the SM group the normal denser character of the marrow was present in some rats on the third and fourth days. The typical conditions on the first through the fourth day are shown in the photographs of sample sections from the marrow of the TCA group (fig. 11 *b* to *c*).

A quantitative study was made of the cell populations with particular reference to myelocytes, neutrophils, nucleated erythroid cells and cells in mitosis. The percentage distributions of the cells were calculated from smears (fig. 10), and the numbers of cells per unit volume and the number of cells in mitosis, from sections (fig. 10). The data on the cell distributions per unit volume given in table 6 were calculated from these counts.

The myelocytes increased significantly in number in the control group on the third day and remained significantly high on the fourth day (fig. 10 and table 6 *B*). In the SM and MCA groups they were less in number than in the control on the first day. On the second day they had decreased in the ECA and TCA groups, remained low in the MCA group and increased in number in the SM group. In all groups there were significant increases in number on the third day, so that the average numbers were equal to that of the control group and remained so on the fourth day.

Normally there are not many myelocytes in mitosis. In the control group there was no change in the number in mitosis throughout the period (fig. 10 and table 6 *C*). In the SM, MCA and TCA groups there were significant decreases in the numbers of these cells in mitosis, and it is thought that mitosis was inhibited. The inhibition of mitosis continued in the MCA group until the fourth day, in the TCA group until the third day and in the SM group until the second day. In the ECA group, in which there had been no change during the first two days, there was a significant decrease on the third day.

The neutrophilic granulocytes increased in number on the third and fourth days (fig. 10 and table 6 *D*) in the control group. In the poisoned groups the numbers were not significantly different from the number in the control group on the first day, but on the second day the numbers in the SM, MCA and TCA groups had been reduced significantly. On the third day the number in the SM group rose significantly, the numbers in the ECA and MCA groups decreased and the number in the TCA group did not change. The levels reached on the third day were characteristic of the fourth day. Thus, in contrast with the control, the poisoned groups suffered a significant decrease in mature neutrophilic granulocytes.

The nucleated erythroid cells did not change significantly in number until the fourth day in the control group, at which time they were significantly fewer than on the first day (fig. 10 and table 6 *E*). In all of the poisoned groups there were significant decreases on the first day and the decreases became more marked on the second day. On the third day the numbers in the SM and ECA groups approached control level, and the numbers in the MCA and TCA groups showed significant increases from second day values. These changes are believed to be

an indication of regeneration of these cells. These trends continued in the SM and MCA groups but slowed up in the TCA group.

There was no change in the number of erythroid cells in mitosis in the control or in the ECA group throughout the period (table 6*F*). In each of the other groups the number of these cells in mitosis was significantly lower than the control average on the first day. The number reached control level in the SM

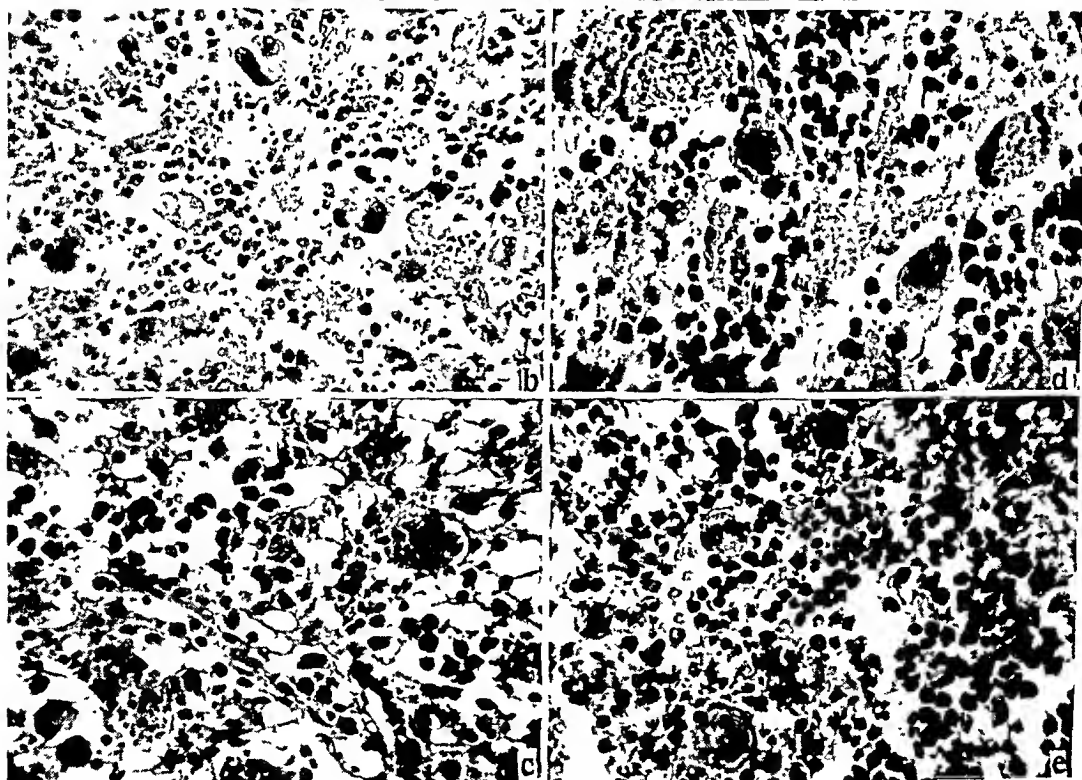
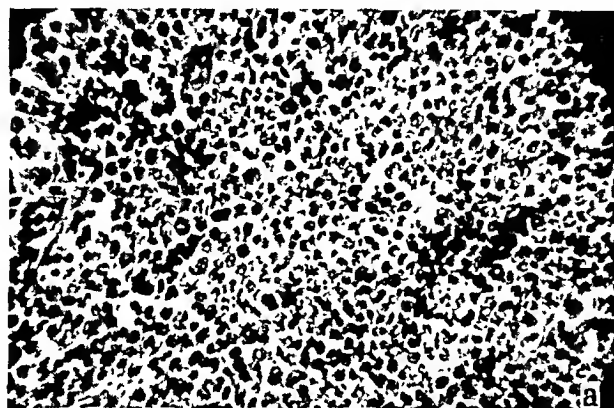


Fig. 11.—Photomicrographs of sections of femoral marrow; hematoxylin and eosin; section 5 microns thick; $\times 280$. (a) Control one day after injection of saline solution. (b, c, d and e) One day, two days, three days and four days, respectively, after injection of tris (2-chloroethyl) amine.

group on the second day, in the TCA group on the third day and in the MCA group on the fourth day. Since the proportionate decrease in the number of cells in mitosis was greater than the decrease in cells, it is believed that

mitosis was inhibited during the first day in all of the poisoned groups except the ECA group.

The initial hypoplasia of the marrow in the poisoned groups appears to be due to decrease in the mitotic activity of the myelocytes and erythroid cells and to continued migration of matured elements. Although there was some destruction of granulocytes, calculated losses of cells could not be accounted for by the

TABLE 6.—*The Femoral Marrow from the Rats Which Were Used for the Study of the Lymphoid Organs*

The average numbers of different types of cells per unit volume (50,000 cubic microns) and the average numbers of myelocytes and erythroid cells in mitosis per 25 unit volumes are recorded as found one, two, three and four days, respectively, after injection. The distributions were calculated from the data in figure 10. Each average is followed by its standard error.

Days	Saline Solution	SM	ECA	MCA	TCA
A. All Types of Cells					
1.....	190 ± 17	115 ± 7*	155 ± 9	162 ± 18	125 ± 13*
2.....	190 ± 15	85 ± 13†	105 ± 33	75 ± 9†	60 ± 8†
3.....	200 ± 5	190 ± 24†	140 ± 9	120 ± 11†	160 ± 10†
4.....	200 ± 8	170 ± 16	140 ± 13	90 ± 11†
B. Myelocytes					
1.....	28 ± 5	14 ± 1*	27 ± 9	15 ± 2*	21 ± 7
2.....	32 ± 4	28 ± 6	16 ± 4	10 ± 1†	11 ± 4
3.....	50 ± 3†	65 ± 7†	39 ± 4†	50 ± 9†	61 ± 13†
4.....	58 ± 3	55 ± 7	57 ± 5	53 ± 5
C. Myelocytes in Mitosis					
1.....	16 ± 2	6 ± 1.7*	16 ± 4	7 ± 1.1*	5 ± 2*
2.....	11 ± 2	15 ± 9	10 ± 5	5 ± 0.3	7 ± 3
3.....	16 ± 2.6	25 ± 2.5	5 ± 0.5	8 ± 2.4	16 ± 1.4†
4.....	19 ± 3	11 ± 1.5†	18 ± 1.7	12 ± 3
D. Neutrophilic Granulocytes					
1.....	45 ± 4	42 ± 3	60 ± 8	66 ± 14	44 ± 8
2.....	47 ± 5	21 ± 2.5†	34 ± 12	26 ± 8†	21 ± 5†
3.....	62 ± 3†	36 ± 3†	23 ± 5	11 ± 2.3	23 ± 8
4.....	60 ± 4	35 ± 5	11 ± 1.4	27 ± 8
E. Nucleated Erythroid Cells					
1.....	100 ± 10	50 ± 3.5*	57 ± 9*	60 ± 7*	33 ± 6*
2.....	87 ± 8	25 ± 3†	40 ± 13	17 ± 1.5†	11 ± 3†
3.....	80 ± 7	66 ± 14†	74 ± 13†	31 ± 2.5†	56 ± 13†
4.....	58 ± 6	67 ± 7	53 ± 8†	22 ± 6†
F. Nucleated Erythroid Cells in Mitosis					
1.....	5 ± 1.4	1 ± 0.4*	3 ± 1.2	1 ± 0.3*	1 ± 0.5*
2.....	6 ± 1.8	5 ± 2.7†	2 ± 2.0	0.4 ± 0.2	0.2 ± 0.2
3.....	5 ± 1.0	5 ± 1.5	4 ± 1.4	0.2 ± 0.2	8 ± 2.3†
4.....	4 ± 0.7	6 ± 2.0	4.0 ± 0.4†	2 ± 0.4

* The number is significantly different from the mean of the first day controls (saline solution).

† The number is significantly different from the mean of the preceding day.

destructive action of the agents. The agents seemed to retard growth and to inhibit mitosis and, as a consequence, the mature cells were not replaced as they migrated from the marrow. The second day seemed to be the peak of the hypoplastic conditions, and recovery was more rapid in the SM group than in the others.

The number of megakaryocytes did not change significantly in any of the groups throughout the period. Hemocytoblasts, mast cells and reticulum cells did not change in number, although there were relative increases in the number of reticulum cells because of the decreases in the populations of the other cells. Eosinophilic granulocytes tended to decrease in number in all of the poisoned groups on the last two days of the period. Plasma cells were always present

and did not seem to be damaged by the agents. The incidence of lymphocytes was small, but they never were numerous even in the control group.

On the whole the marrow did not undergo great destructive changes such as occurred in the lymphoid organs as a result of the intoxication following injection of vesicants. In both lymphoid organs and marrow there was cellular hypoplasia, but in the former a large part of the hypoplasia was due to injury of cells, whereas in the latter most of the hypoplasia was apparently caused by migration of matured elements without replacement. The erythroid elements of the marrow seemed to be depleted to a greater degree than were the myeloid elements.

It has been stated by Karnofsky and associates²⁰ that the cells of the marrow swell in rats poisoned with methyl-bis (2-chloroethyl) amine. This statement is hard to prove quantitatively since fixation changes may have some effect on the physical characteristics of injured cells. However, in order to test this view, camera lucida drawings were made under oil immersion magnification (1,700 diameters) of the largest of the several types of cells chosen at random from a number of fields in smear preparations. These drawings were then measured and the average diameters recorded. The average diameter of the largest hemoblasts in the control group of the first day was 13.8 microns. The average diameter of the largest hemoblasts in each group except the SM group exceeded that of the control. The average diameter of the largest myelocytes in the control group of the first day was 14.5 microns. This diameter was exceeded by the largest myelocytes in each of the poisoned groups on the first and later days. The diameters of the viable neutrophilic and eosinophilic granulocytes and that of the erythroblasts did not exceed the diameters of these cells in the control. Hence it is concluded in agreement with Karnofsky and associates²⁰ that swelling of the hemoblasts and myelocytes is characteristic of the marrow of rats injured by injected vesicants. This swelling may be the prelude to death, for at the end of the first day there were significantly fewer myelocytes in the SM and MCA groups than in the control, and on the second day, in the ECA and TCA groups. Furthermore, in the swollen cells the nuclei did not seem to be as sharply defined as those of the cells of normal size.

In the ECA group practically no degenerated cells were observed. But in the other groups, particularly in the MCA group, there was marked evidence of nuclear degeneration in all types of cells on the first and second days after injection. As in the degenerating lymphocytes of the lymphoid organs, the chromatin of the nucleus was concentrated in discrete small opaque dense basophilic masses, and there was no sign of a nuclear membrane. In the larger myelocytes, the chromatin was massed into irregular strands, and the nuclear membrane was broken. Many of these degenerated cells remained free, but some of them had been ingested by macrophages of reticulum cell origin. A peculiar kind of degeneration was noted in the neutrophils of the SM group on the third day. The nucleus appeared as an amorphous mass of chromatin with bleblike bulbs protruding from the margin. Some of the megakaryocytes contained pyknotic nuclei, and in a few of them the nucleus had disintegrated.

An analysis of the distributions of the myeloid cells of the marrow and of the neutrophils of the circulating blood shows that there is a definite relation between the number of myelocytes and their mitotic activity and the number of neutrophils present in the blood. None of the agents seemed to cause the same degree of effect, although the character of the changes was the same. Methyl-bis (2-chloroethyl) amine caused the greatest change in that an initial decrease of myelocytes associated with continued inhibition of mitosis was accompanied by continued and marked decrease of the neutrophils of the blood and marked neutropenia of the

bone marrow. In the SM and TCA groups, where the inhibition of the mitosis of the myelocytes did not last longer than one day, there was only temporary neutropenia of the blood on the day following the inhibition of mitosis; in both of these groups, however, this was accompanied by continued decrease in the number of neutrophils of the marrow. In the ECA group, where the inhibition of mitosis was slow to appear, there was no change in the neutrophils of the blood. Thus methyl-bis (2-chloroethyl) amine seems to be the only agent which had a marked effect both on the number of neutrophils of the blood and on the myeloid elements of the marrow.

In all groups except the ECA group there were marked decreases in the numbers of nucleated erythroid cells and inhibition of mitosis of these cells for at least one day. It is thought that much of the hypoplastic appearance of the marrow was due to the retardation of the mitotic activity of these cells. Since there were many masses of mature red blood corpuscles in the sinuses, it is possible that maturation of the cells continued, but the cells were not replaced as they matured. There seems to be no doubt that anemia would eventually occur in these rats. When the mitotic activity of the erythroid cells was resumed, there was no evidence that these cells were proliferating from the endothelial cells of the sinusoids, but the islands of erythroid cells appeared to arise from erythroid cells which had remained uninjured in the intersinusoidal reticulum spaces.

COMMENT. ON THE BONE MARROW

The percentage distributions of the cells of the bone marrow of the control rats were like those described by Stasney and Higgins,⁴⁸ Kindred¹ and Töppner,⁴⁹ and the distributions of cells per unit volume agreed with those given for the rat.⁵⁰ Study of the bone marrow of men gassed with sulfur mustard in World War I showed that there was aplasia in those with the greatest neutropenia of the circulating blood. The neutrophils had been swept out, and there was peripheral hyperplasia of myeloid cells indiscriminately arranged.^{2a} In rabbits given injections of sulfur mustard there was aplasia four days after injection, with decrease in mitosis of the remaining cells. These degenerative changes were followed, even after a second injection of sulfur mustard, by regeneration of all types of marrow cells and increase of mitosis.^{3a} In reports of recent investigations, degenerative changes of the marrow have been described as following injection of, or exposure to, sulfur mustard,⁵¹ ethyl-bis (2-chloroethyl) amine (very slight change),^{44c} methyl-bis (2-chloroethyl) amine⁵² and tris (2-chloroethyl)

48. Stasney, J., and Higgins, G. M.: *Anat. Rec.* **63**:77, 1935.

49. Töppner, R.: *Folia haemat.* **66**:48, 1942.

50. Farrar, G. E., Jr.: *Am. J. Physiol.* **117**:662, 1936. Kindred.¹

51. (a) Salter, W. T.; Bullock, T. H., and Fishman, J. B.: Unpublished data, 1943. (b) Cameron, G. R.: Unpublished data, 1942.

52. Cameron, G. R., and Foss, G. I.: Unpublished data, 1942. Karnofsky and others.²⁹ Cameron and Short.^{44b} Graef and others.^{44c} Crawford and Smith.^{45a} Salter and others.^{51a}

amine.⁵³ Tris (2-chloroethyl) amine was most toxic for rats when administered by gassing or by subcutaneous injections; when the agents were injected intravenously, sulfur mustard had the most toxic effect.⁵⁴ In the present investigation, in which the agents were intravenously injected, tris (2-chloroethyl) amine was found to be the most toxic, but methyl-bis (2-chloroethyl) amine had the longest depressive effect. The effects of these agents do not seem to be as damaging to the cells as roentgen radiation,⁵⁵ since there was not as much cell necrosis and phagocytosis. Mitosis of the myeloid cells is inhibited temporarily, but the number of neutrophils seems to be decreased by migration rather than by destruction. It is possible that the neutrophils that were on the way to maturity were stimulated to more rapid maturation, whereas those which were growing were inhibited. The latter view rests on the observation that young myelocytes were diminished in number in the first two days after injection and increased during the last two. Hyperemia of the marrow is characteristic, and this was also observed in mice exposed to roentgen rays.⁵⁶

The myelocytes show reduction in number and evidence of injury after being exposed to methyl-bis (2-chloroethyl) amine.⁵⁶ They are destroyed by roentgen rays in mice⁵⁷; they are decreased in absolute number in the rat⁵⁸ and are the cells particularly affected by roentgen rays.⁵⁹ These observations are in agreement with the initial changes observed in the myelocytes after injection of the vesicants. Mitosis, it has been concluded in the present investigation, was initially inhibited in the rats injured by injected vesicants; it has been observed by others to be inhibited as a result of injury produced with mustard,⁶⁰ with methyl-bis (2-chloroethyl) amine²⁹ and with roentgen rays.⁶¹ Mature neutrophilic granulocytes, which decreased in number on the second day after injection and continued to decrease in the marrow of the poisoned rats, and which in the MCA group showed greater injury than in the other groups, have been found to react in the same manner to methyl-bis (2-chloroethyl) amine by Karnofsky and associates.²⁹ They decreased in rabbits after injection of sulfur mustard^{3a} and in men after exposure

53. Graef and others.^{44c} Short.^{46a} Hanck.^{46b}

54. Dougherty, T. H.; Goodman, L. S.; Gilman, A., and Dougherty, J.: Unpublished data, 1942.

55. Heineke, H.: *Deutsche Ztschr. f. Chir.* **78**:196, 1905. Dunlap.¹⁷ Warren and Whipple.^{43a}

56. Karnofsky and others.²⁹ Cameron and Short.^{44b}

57. Warthin, A. S.: *Internat. Clin.* **4**:243, 1906.

58. Lingley, J. R.; Gall, E. A., and Hilcken, J. A.: *Am. J. Path.* **16**:845, 1940.

59. Ssipowsky, P. W.: *Beitr. z. path. Anat. u. z. allg. Path.* **94**:1, 1934.

60. Krumbhaar.^{2a} Pappenheimer and Vance.^{3a}

61. Mottram, J. D.: *Arch. Radiol. & Electrotherap.* **25**:197, 1920. Warthin.⁵⁷

to this vesicant.^{2a} They showed no change in number in mice during the first forty-eight hours after being exposed to roentgen rays but were gradually reduced in number after this time.⁶²

Erythrogenic cells, which are more resistant to the effects of the vesicants than are the myeloid cells, have shown injury only after being exposed to roentgen rays for a long time.⁵⁸ The megakaryocytes, which have been shown here to exhibit some signs of injury but no reduction in number, are reported to react in the same way to methyl-bis (2-chloroethyl) amine²⁹; with sulfur mustard they are reduced in number for some time after the initial injection.^{3a} Following roentgen irradiation they are said to be damaged and reduced in number.⁶³ Eosinophils which were reduced in number in the poisoned rats during the last two days of the four day period have been observed to be injured forty-eight hours after injection with methyl-bis (2-chloroethyl) amine.²⁹ Thus, in general the effects of the vesicants on the several stages of development of the white and red cells of the marrow are the same as those produced by roentgen rays, and except for the injuries caused by methyl-bis (2-chloroethyl) amine, are not as drastic.

In the normal rat the marrow's production of mature granulocytes has been calculated to be sufficient to supply about fifty-eight times as many granulocytes per hour as are needed by the circulating blood to maintain the average count.¹ Hence it appears that even if this source were temporarily inhibited by the vesicants, there would be no immediate neutropenia of the blood. It is possible that if the toxic action continued, neutropenia would occur. In the table showing the distributions of the neutrophils of the blood (table 1, C), it will be seen that only in the MCA group was there a trend toward neutropenia, and in this group there was more destruction of the myeloid cells of the marrow than in the other groups.

According to Dougherty and White,^{21b} no extensive degeneration of the myeloid elements was seen in mice given injections of pituitary adrenocorticotrophic hormone, although some degenerated lymphocytes were observed in macrophages. In view of these observations a limited quantitative study was made of the femoral marrow of adrenalectomized rats that received injections of 0.3, 0.4 and 0.5 mg. of tris (2-chloroethyl) amine per kilogram of body weight. In the marrow of these rats there was a tendency toward hyperemia and hypoplasia at the end of the first day after injection in those rats receiving the largest amount of the agent. There were no changes in the proportions of the different types of cells. Such a condition obtained in nonadrenalectomized rats on the

62. Lawrence and Tennant.²⁶ Warren and Whipple,^{43a} Warthin,⁵⁷ Lingley and others.⁵⁸

63. Shouse and others.²⁴ Heineke,⁵⁵ Ssipowsky.⁵⁹

first day after injection of 1.0 mg. of tris (2-chloroethyl) amine per kilogram of body weight. On the second day after injection the hyperemia and the hypoplasia were characteristic of all groups. Many of the neutrophils were injured, but no injured lymphocytes were observed. There were large edematous spaces between the sinusoids. The myeloid cells were scattered, and in them mitosis seemed to be as in the normal myelocytes. A few macrophages were observed. The most notable characteristic was the practical absence of erythroblasts and normoblasts. The megakaryocytes were prominent, and their nuclei were swollen. The percentages of the neutrophils were significantly less than in the control group (starved adrenalectomized rats), and there was increase in the relative proportions of myeloblasts and myelocytes. These conditions continued throughout the four day period in the rats which survived. These observations supplement the data obtained from the study of the lymphoid organs of these adrenalectomized rats in support of the view that the agent acts directly on the cell and its effect is not mediated by the adrenal gland.

SUMMARY

In young adult albino rats given single intravenous injections of lethal doses of sulfur mustard (bis [2-chloroethyl] sulfide) and the nitrogen mustards (ethyl-bis [2-chloroethyl] amine, methyl-bis [2-chloroethyl] amine and tris [2-chloroethyl] amine) the following major changes occurred in the circulating blood and the hemopoietic organs: (1) lymphopenia in all groups; (2) lymphopenia and neutropenia in the group given methyl-bis(2-chloroethyl) amine; (3) reduction of the weight and the amount of the lymphoid tissue of the thymus, the cervical lymph nodes and the spleen, and (4) hypoplasia and hyperemia of the femoral marrow.

Tris (2-chloroethyl) amine caused the greatest lymphopenia and had the most degenerative effect on the lymphocytes of the thymus, the lymph nodes and the spleen. Next in order of toxicity were the ethyl-bis and methyl-bis (2-chloroethyl) amines, which produced almost identical degenerative effects on these organs. Sulfur mustard was least effective initially but caused terminal changes of the same degree as those produced by the nitrogen mustards.

In the lymphoid organs the lymphoid tissue shrinks in volume because of the destruction of lymphocytes, the inhibition of mitosis of the medium-sized lymphocytes and the migration of lymphocytes which go into the blood stream. The reticulum cells of the lymphoid organs are not injured directly by the agents. These cells as fixed phagocytes ingest and digest the degenerated lymphocytes. It is believed that many of them perish from the exhaustion resulting from their unusual phagocytic activity. Regeneration of the lymphoid tissue

begins on the third day after injection, in general, and is characterized by increase of number and mitotic activity of the medium-sized lymphocytes. It is believed that these cells arise from small lymphocytes which have escaped the toxic action of the agents.

In adrenalectomized rats given intravenous injections of tris (2-chloroethyl) amine the same degenerative changes occurred as were observed in the nonadrenalectomized rats. Hence it is believed that the damage done to the lymphocytes in the lymphoid organs of the vesicant-injured rats is caused by direct action of the agents. Further evidence for the theory that the nitrogen mustards act directly on the lymphocytes accrues from the *in vitro* study of fragments of thymus suspended in plasma and tris (2-chloroethyl) amine. In these preparations the lymphocytes showed the same karyorrhectic changes which characterized them in the lymphoid organs of rats given injections of vesicants.

There is a general direct correlation between the decrease of the number of lymphocytes of the blood and the degeneration of the lymphoid organs. However, it is thought that the initial loss of lymphocytes of the blood stream results because the lymphocytes migrate through the degenerated epithelium of the intestine into the lumen.

The bone marrow reacted more slowly to the vesicants than did the lymphoid organs, but it became hypoplastic and hyperemic. There was some destruction of cells, particularly of the mature granulocytes. The most marked effects were the decrease of the number of mature granulocytes and the inhibition of the mitosis of myelocytes lasting one day. Regeneration of myelocytes began on the third day after injection, but their maturation was apparently inhibited except in the sulfur mustard group. Methyl-bis (2-chloroethyl) amine had a more depressing effect on the myeloid cell population than did the other agents. Ethyl-bis (2-chloroethyl) amine had a slight effect.

The nucleated erythroid cells of the marrow decreased markedly in number, and their mitosis was inhibited. Regeneration began in these cells on the third day, and the cells which were dividing appeared to arise from erythroblasts which had escaped injury by the agents.

The megakaryocytes seemed to suffer the least change of any of the hemopoietic cells. No phagocytes containing great masses of degenerated cells were seen in the bone marrow, and in this respect there was a difference between the degenerative changes of the lymphoid organs and those of the marrow.

The degenerative changes of the hemopoietic organs as a whole resembled more closely those produced by roentgen rays than those caused by any other agents.

ACUTE AND CHRONIC TOXICITY OF METHYL CHLORIDE

IV. Histopathologic Observations

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AND

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PRECEDING reports deal with the properties of methyl chloride, the method of exposure, and the symptoms¹ and the mortality resulting in animals exposed to concentrations ranging from 4,000 to 300 parts per million,² and with the results of hematologic and biochemical studies.³ Histopathologic material derived from these experiments will be described in this communication.

LITERATURE

Autopsy findings in fatal human methyl chloride poisoning have been reported by Schwarz,⁴ Kegel, McNally and Pope⁵ and McNally.⁶ Sayers and his associates⁷ have made an extensive study of guinea pigs, and further observations on this species have been reported by White and Sommers⁸ and Schwarz.⁴ Schwarz has also reported findings in mice and rabbits, and Montmartini⁹ findings in dogs. The principal abnormalities reported by these authors were: congestion, edema and hemorrhage in various organs, particularly the lungs; fatty changes in

From the Pathology Laboratory (Robert C. Dunn) and the Industrial Hygiene Research Laboratory (Willie W. Smith), National Institute of Health, United States Public Health Service.

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4. Schwarz, F.: *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **7**:278, 1926.

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7. Sayers, R. R.; Yant, W. P.; Thomas, B. G., and Berger, L. B.: *Physiological Response Attending Exposure to Vapors of Methyl Bromide, Methyl Chloride, Ethyl Bromide and Ethyl Chloride*, Public Health Bulletin 185, United States Treasury Department, Public Health Service, 1929.

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the liver; degenerative changes in the kidneys and the liver, and degeneration of anterior horn cells.⁴ No mention was made of necrosis of renal cortical tubules occurring in mice and rats, or of hemoglobinuria and associated conditions in mice, which were observed with great frequency in the present study.

METHODS

Seven species of animals were exposed to concentrations of methyl chloride ranging from 4,000 to 500 p.p.m. for six hours a day six days a week until death. The eighth species studied was exposed to 11,000 p.p.m. for two hours a day on two consecutive days.

Organs were fixed in 4 per cent solution of formaldehyde buffered to pH 7. Paraffin sections were stained routinely with eosin-polychrome methylene blue, prussian blue (used as a reagent for the detection of iron) and the hemoglobin-collagen stain.¹⁰ Frozen sections were stained by the oil red O-isopropyl alcohol technic.¹¹ Brain and spinal cord were fixed in Orth's solution.

OBSERVATIONS

Mice.—Mice of three strains, 3 to 4 months old and equally distributed as to sex, were exposed to 2,000 p.p.m. Except for 5 mice of one strain (C₃H), the animals died during the week of exposures. Litter mate controls showed no significant lesions.

The evidences of injury of the kidneys, fat, necrosis, hemoglobin globules and hemoglobin casts are shown in the accompanying table. The epithelium of scattered convoluted tubules showed various degenerative changes, grading into

*Renal Changes Observed in Mice Exposed to Methyl Chloride
2,000 Parts per Million*

	Swiss Strain				Strain A				Strain C ₃ H			
	Degree of Change				Degree of Change				Degree of Change			
	Mice	Slight	Mod- erate	Marked	Mice	Slight	Mod- erate	Marked	Mice	Slight	Mod- erate	Marked
Fat.....	9	2	2	0	15	3	3	0	15	2	6	0
Necrosis.....	25	7	11	0	15	4	9	1	15	5	6	0
Hemoglobin globules....	25	0	1	2	15	2	7	6	15	0	4	0
Hemoglobin casts.....	25	1	3	1	15	4	5	0	15	2	3	6

necrosis and fibrocellular replacement scars infiltrated by a few to moderate numbers of polymorphonuclear neutrophils. In the earlier stages of this process epithelial cells of a few to many convoluted tubules and occasionally the thick portion of Henle's loop displayed pyknotic nuclei and fibrillar basophilic cytoplasm. In the cortex of some kidneys in which necrosis was present there were radially arranged elongated areas of regeneration in which tubules were lined by large basophilic epithelial cells, which occasionally showed mitosis. Hemoglobin globules were often found in the cytoplasm of cells lining the convoluted tubules. In addition to the hemoglobin casts there were occasionally small numbers of eosinophilic hyaline or basophilic fibrillar casts.

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There were slight to moderate fatty changes in the kidneys of about half of the animals in each group, the fat finely globular and predominantly in the epithelial cells of the convoluted tubules. Occasionally a kidney showed a few altered glomeruli, the changes consisting of shrunken or very cellular glomerular tufts, hyaline masses in the lumens of capillaries of tufts, hyalinized capillary walls, or basophilic fibrillar debris in the capsular space. In many mice, at autopsy the urine of the bladder was reddish, gave a strongly positive reaction in the benzidine test for hemoglobin and contained many reddish granular casts.

In all but a few mice the hepatic cells in the centrolobular areas of the liver showed a moderate degree of fatty metamorphosis. There was moderately extensive necrosis in the centrolobular areas in 4 of these mice. Congestion was the only pulmonary change noted with any regularity, though a slight degree of edema was noted rarely, and hemorrhage in occasional alveoli. Occasionally myocarditis was seen but was probably infectious.

Rats.—In general the pathologic changes seen in the kidneys of rats were similar to those seen in the kidneys of mice, with the exception of the findings associated with hemoglobinuria. In all of a group of 10 rats dying after the fourth or fifth exposure to 4,000 p.p.m., and in 8 of the 16 rats dying after the fifth exposure to 3,000 p.p.m., the kidneys showed few to moderately numerous necrotic convoluted tubules. Half of a group of 12 rats 4 months old exposed to 2,000 p.p.m. for two weeks died and showed the cortical necrosis described; those that were killed, and the 11 litter mate controls, showed none. Occasionally small amounts of fat appeared in the epithelial cells of the convoluted tubules.

Small to moderate amounts of fat were frequently seen in hepatic cells, and centrolobular necrosis was occasionally noted in the liver. In the lungs congestion and, rarely, a slight degree of edema were the only pathologic changes which were not also encountered in unexposed rats. Occasionally myocarditis, apparently infectious, was noted. Rats exposed for six to nine months to 1,000 and 500 p.p.m. showed no significant changes.

Guinea Pigs.—Unlike mice and rats, guinea pigs showed neither renal necrosis nor hemoglobin deposits. However, fatty metamorphosis of moderate to marked degree was noted in both kidney and liver in 7 of 14 guinea pigs dying after one to three daily exposures to 3,000 or 2,000 p.p.m. These fatty changes were observed chiefly in the epithelial cells lining the convoluted tubules and the thick portion of Henle's loop and in hepatic cells in the centrolobular areas. Of 31 animals exposed to 1,000 p.p.m., fatty metamorphosis was present to a slight degree in 12 and to a moderate to marked degree in 4 others. In the same animals the liver showed moderate to marked amounts of fat.

In the lungs of guinea pigs dying within the first few days of being exposed to 3,000 or 2,000 p.p.m., marked congestion and edema were usually observed, while after exposures to lower concentrations of methyl chloride there was little change.

Occasional animals showed basophilic renal casts, traces of hemosiderin in the hepatic and Kupffer cells, and myocarditis. The adrenal glands of 4 animals showed no change. Guinea pigs surviving nine months of exposure to 500 p.p.m. showed no pathologic changes.

Dogs.—Fatty changes of the liver and the kidney and renal necrosis were minimal or absent. In 1 of 6 dogs exposed to 3,000 p.p.m. the urine was reddish brown and strongly reactive with benzidine, and there were a few small granular hemoglobin casts in the kidney. Hemosiderin was found in small amounts in renal, hepatic and Kupffer cells, and in large amounts in the spleen. In other dogs of

this group there was slight to marked splenic hemosiderosis, but hemosiderin was not found in the other organs and hemoglobin casts and hemoglobinuria were absent, although hemoglobinuria was occasionally noted in other dogs just before death.³ In dogs exposed to 2,000, 1,000 and 500 p.p.m., hemosiderin was usually found in moderate to large amounts in the spleen and often in moderate amounts in the Kupffer cells of the liver and in the epithelium of the convoluted tubules of the kidney.

The lungs of dogs exposed to 3,000 p.p.m. often showed marked congestion, and in an occasional dog there were small amounts of blood in scattered alveoli or a moderate number of hemosiderin-laden macrophages in the alveolar septums. Adrenal glands, stomach, pancreas, ovaries and heart appeared unchanged.

The brains and spinal cords of 5 pups, in all of which had developed severe neuromuscular symptoms¹ after exposures ranging from one week at 2,000 p.p.m. to thirty weeks at 500 p.p.m., were examined by Dr. R. D. Lillie who noted gross and microscopic lesions which appeared to be of an infectious nature and from which possible toxic morphologic changes could not be dissociated. Similar lesions were observed in an unexposed litter mate.

Rabbits.—Most rabbits exposed to 4,000 p.p.m. methyl chloride died after the second week of exposures, and those exposed to 2,000 p.p.m., after four to five weeks. Pulmonary congestion, often accompanied by edema, was frequently seen in rabbits dying in the first week but not in those dying thereafter. Many of the rabbits showed pneumonia, possibly as a secondary effect of the exposures. The only other changes of significance were found in a rabbit dying after twenty-seven weeks of exposures to 500 p.p.m. and included moderate fatty metamorphosis of the liver, the kidney and the heart, and extensive coagulation necrosis of the centrilobular areas of the liver. Three rabbits dying after exposures to 4,000 p.p.m. and 2 others killed after two weeks of exposures to 3,000 p.p.m. showed no significant changes. Chronic myocarditis in 1 animal proved to be an extension from the adjacent suppurating lung. No pathologic changes were observed in brain, spinal cord or sciatic nerve.

Cats.—In a cat which became moribund and was killed in the third week of being exposed to 2,000 p.p.m., the lungs were moderately congested, macrophages in the alveolar septums contained moderate amounts of hemosiderin, and hepatic cells contained many fine to coarse fat globules diffusely distributed. There were similar findings in 2 other cats that died in the fifth week of exposures.

Monkeys.—A single monkey, dying after sixteen weeks of exposures to 500 p.p.m., was examined for microscopic changes. Other than a fairly marked hemosiderosis of spleen and Kupffer cells, with traces of hemosiderin in hepatic cells, all organs examined (lungs, liver, kidneys, heart and spleen) appeared to be normal.

The brain of a monkey that died in the second week of exposures to 2,000 p.p.m., and the brains and the spinal cords of 2 others that were exposed intermittently to that concentration over periods of four and nine weeks, respectively, were examined by Dr. R. D. Lillie, who observed no pathologic changes distinctly attributable to methyl chloride.

Goats.—Grossly, the only pathologic changes in 2 goats exposed for two hours a day on two consecutive days to 11,000 p.p.m. were petechial hemorrhages in the small intestine and the adjacent mesentery. In the one goat the kidney had a mottled and hyperemic appearance and the lungs were hyperemic with a small amount of foamy mucus in the bronchi; in the other the lungs were very hyperemic

and appeared to be slightly edematous. These observations were confirmed microscopically, with the addition that moderate numbers of fat globules, mostly fine, were diffusely distributed in the hepatic cells.

SUMMARY

On the basis of a study of several hundred animals, the majority mice, rats and guinea pigs, with fewer dogs, rabbits, cats, monkeys and goats, the only morphologic changes that appear to be a direct result of inhalation of methyl chloride are variable degrees of necrosis of the convoluted tubules of the kidneys in mice and rats, renal changes associated with hemoglobinuria in mice and occasional dogs, and a fairly constant but low to moderate amount of fatty metamorphosis of the liver and kidneys in the smaller species of animals studied. Clinically, pulmonary edema was noted frequently and appeared to be a direct result of the irritation due to inhalation of methyl chloride. Although focal myocardial changes and pneumonia were occasionally noted, they were often closely associated and did not appear to result directly from the inhalation of methyl chloride.

HISTOLOGIC COMPARISON OF THE BRAINS OF VITAMIN A-DEFICIENT AND VITAMIN E-DEFICIENT CHICKS

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AS POINTED out by Moore,¹ considerable evidence has been accumulated indicating a definite synergistic relation between the vitamin A and the vitamin E of a ration. This is shown by the defective storage of vitamin A in animals deficient in vitamin E and also by the effect of vitamin E on the growth response of animals receiving vitamin A. It is possible, therefore, that certain reactions in experimental animals which have been attributed to vitamin E deficiency may also be related to an unsuspected vitamin A deficiency. A case in point is encephalomalacia occurring in the chick. Under conditions of vitamin E deficiency a characteristic pathologic syndrome develops which is marked by a typical histologic picture of brain degeneration (Pappenheimer and Goettsch²; Adamstone³). Extensive studies of other phases of this condition have been made by Pappenheimer, Goettsch and Jungherr,⁴ as well as by Dam,⁵ Dam and Glavind,⁶ Bird⁷ and others. Patrick and Morgan⁸ presented evidence that vitamin A deficiency may arise spontaneously in vitamin E-deficient diets which have produced encephalomalacia and suggested that so-called field encephalomalacia may be in reality a vitamin A deficiency disease. Moreover, in experiments in which young chicks were reared on diets deficient in vitamins A and E, respectively, the reactions produced were similar in both cases. Hence, since diagnosis is difficult, a careful histologic study of the two conditions is highly desirable in order to determine whether they can be distinguished with certainty by histologic methods and also whether the findings support the suggested synergistic relation between vitamins A and E. The present report gives the results of a study carried out with this object in view.

From the University of Illinois.

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MATERIALS AND METHODS

For the purposes of these investigations groups of newly hatched chicks were reared in wire-floored brooder pens on rations designed to produce vitamin A and vitamin E deficiency. The following diets were employed:

(1) Basal Ration—Control	(2) Vitamin A-Deficient Ration
Ground yellow corn..... 46	Ground white corn..... 52
Wheat bran..... 13	Wheat bran..... 13
Wheat middlings..... 13	Wheat middlings..... 13
Alfalfa meal..... 5	Meat scrap..... 10
Meat scrap..... 10	Dried skim milk..... 10
Dried skim milk..... 10	Salt 1
Salt 1	—
Cod liver oil..... 2	99
—	100 Cod liver oil..... 0 to 1 per cent

(3) Iron-Treated Vitamin E-Deficient Ration: Ration 1 treated with ferric chloride dissolved in ether (Adamstone³).

(4) Ration Deficient in Vitamins A and E: Ration 2 rendered E deficient by treatment with ferric chloride.

(5) Purified Ration—E Deficient. This was made up as follows:

Casein 250 Gm.	Salts 45 Gm.
Dextrin 500 Gm.	L. Cystine..... 3 Gm.
Sucrose 100 Gm.	—
Lard 102 Gm.	1,000

Supplements per Thousand Grams of Ration 5

Thiamine hydrochloride..... 5 mg.	Choline chloride..... 1,000 mg.
Riboflavin 10 mg.	Biotin 2 mg.
Pyridoxine 5 mg.	Vitamin A..... 50 U.S.P. units
Nicotinic acid..... 100 mg	Vitamin D..... 5 U.S.P. units
Calcium pantothenate..... 15 mg.	Vitamin K..... 5 mg.
p-aminobenzoic acid..... 150 mg.	Yeast 5 per cent
Inositol 400 mg.	Gelatin 10 per cent

Summary of Experiments

Lot	Vitamin Deficiency	Supplement *	Chicks	Chicks Showing E-Deficiency Lesions of Brain			
				Chicks Showing Microscopic Lesions	Gross Lesions	Microscopic Lesions	
1	Control	2% C.L.O.	15	0
2	A	U.V.L.	20	20	10
3	A	¼% C.L.O.	15	15	10
4	A	½% C.L.O.	15	0
5	A	1% C.L.O.	15	0
6	A, E	2% C.L.O.	20	20	10	..	2
7	E	2% C.L.O.	20	12	11	12	12
8	E	0.4% H.L.O.	18	7	5	7	7
9	E	Synthetic	20	3	..	3	3

* C.L.O. means cod liver oil; H.L.O., halibut liver oil; U.V.L., ultraviolet rays, given twenty minutes daily.

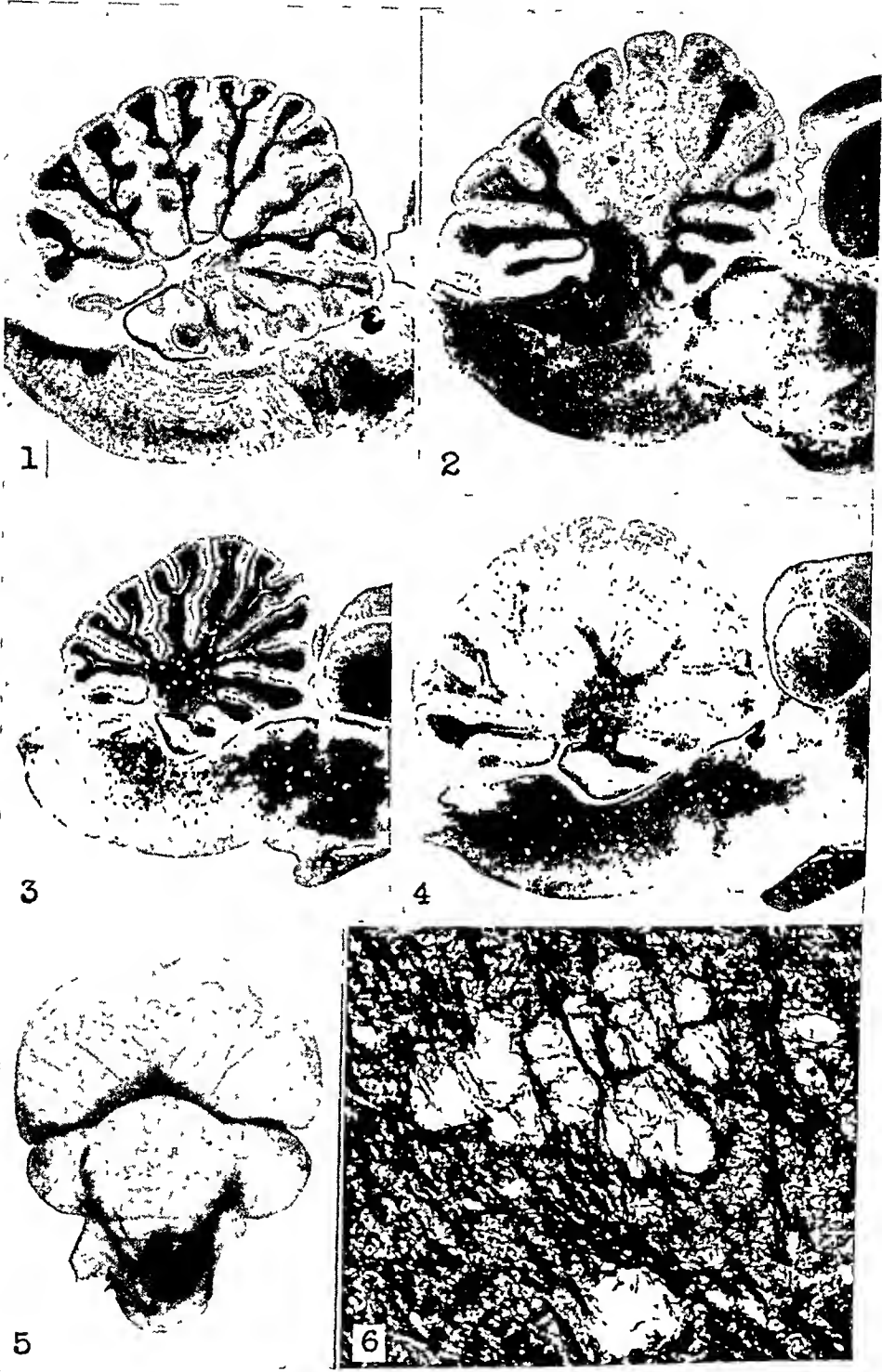
The experimental program based on use of the described diets is outlined in the accompanying table. A brief explanation of the plan of the experiment is necessary. In lots 2, 3, 4 and 5 the vitamin A-deficient diet (ration 2) was used. In lot 2 the chicks were exposed to ultraviolet rays for twenty minutes daily in order to protect them against vitamin D deficiency. In lots 3, 4 and 5, different supplements of cod liver oil were used in order to find the level at which the birds were completely protected from A deficiency. In lot 6 the vitamin A-deficient ration 2 was treated with ferric chloride dissolved in ether in order to superimpose a vitamin E deficiency as well. For lots 7 and 8 the iron-treated vitamin E-deficient ration was employed, but halibut liver oil was used in lot 8 in place of cod liver oil. In lot 9 the birds were fed the purified ration supplemented with the synthetic vitamins listed.

As the experiment progressed the chicks were watched carefully for signs of imbalance so that a sufficient number might be killed before death in order to secure well fixed brain material. From the data given in the table it will be seen that symptoms of imbalance developed in all birds in lots 2 and 3. A total of 20 brains were secured from them. All the chicks in lots 4 and 5 were apparently completely protected and showed no signs of incoordination. In lot 6 imbalance developed in all birds, and from these birds 10 brains were obtained. In lots 7 and 8 there were some chicks showing imbalance in spite of the rather high amounts of cod and halibut liver oil with which the diet was supplemented in those experiments. Finally, in lot 9 there were a few more birds showing incoordination.

The brain of each affected chick was quickly removed at autopsy after decapitation and fixed in a 4 per cent solution of formaldehyde or in 95 per cent alcohol. Later it was sectioned at 6 to 10 microns and the slides were stained with Heidenhain's iron-alum-hematoxylin or with cresyl violet. For this study the brains of the E-deficient birds were supplemented with a great deal of material secured earlier in other similar experiments.

CLINICAL SYMPTOMS OF VITAMIN A AND VITAMIN E DEFICIENCY IN THE CHICK

Both A and E deficiency in the chick are characterized by severe locomotor disturbances having their origin in the central nervous system. In both there are definite incoordination and imbalance, making the two conditions difficult to distinguish. In general the following differences may be observed, but they are not sufficiently cleancut to be reliable. Chicks deficient in vitamin A are usually considerably smaller than those deficient in vitamin E if they are of comparable age, and in A-deficient birds the characteristic incoordination develops gradually. When the condition is well established the chicks usually stand hunched and unsteady, and if disturbed, they walk in an uncertain and irregular manner. Eventually the affected birds are prostrated and lie still, but if set on their feet, they fall quickly, often without attempting to walk. In E-deficient birds the incoordination usually appears with great suddenness, and the larger and more rapidly growing birds are often the first to be affected. These birds are also inclined to mope, balancing themselves somewhat unsteadily. If disturbed, they usually run and



FIGURES 1 TO 6
(See legends on opposite page)

stagger rapidly across the pen. When prostrated they lie with legs outstretched, wings spread out and head retracted. Often, when disturbed, they execute "bicycling movements" with their legs. A coarse tremor is evident throughout the body—this reaction being more violent than in A-deficient chicks. Obviously it is difficult to distinguish vitamin A and vitamin E deficiency in chicks on the basis of the clinical symptoms described, and one must rely on a careful histologic examination of the brains.

GROSS APPEARANCE OF BRAINS

In contrast to brains of vitamin E-deficient chicks, the brains of vitamin A-deficient birds show no visible gross lesions of any sort when cut longitudinally. Their brains are, however, smaller than those of normal birds of the same age because of the slower growth (compare figs. 1 and 3). The brains of E-deficient birds show distinct regions of hemorrhage or areas of greenish yellow discoloration (fig. 5). These lesions are sometimes hidden because the surface tissues are unaffected, but they are readily recognized in longitudinal sections. Another characteristic feature of E-deficient brains is the common occurrence of edema, which may be so pronounced that fluid escapes in considerable quantity when the tissues of the brain are cut.

Although the brains of vitamin A-deficient chicks showed no conspicuous gross lesions, examination of longitudinal sections with a hand lens revealed numerous pinpoint areas of degeneration in certain areas (fig. 3). These were most frequently seen in the brain stem; the optic chiasma, the base of the cerebellum and, in a few rare cases, the cerebral hemispheres.

Birds receiving vitamin E-deficient diets showed typical degenerate areas characteristic of nutritional encephalomalacia in the cerebellum (figs. 2, 4 and 5) or, more rarely, in the cerebral hemispheres. Occasionally, small pinpoint lesions similar to those found uniformly in A-deficient brains were to be seen in supposedly E-deficient brains (fig. 4). It would appear, therefore, that an incipient condition of A deficiency was present in birds in which such lesions occurred. This

EXPLANATION OF FIGURES 1 TO 6

Fig. 1.—Longitudinal section of the cerebellum of a normal chick.

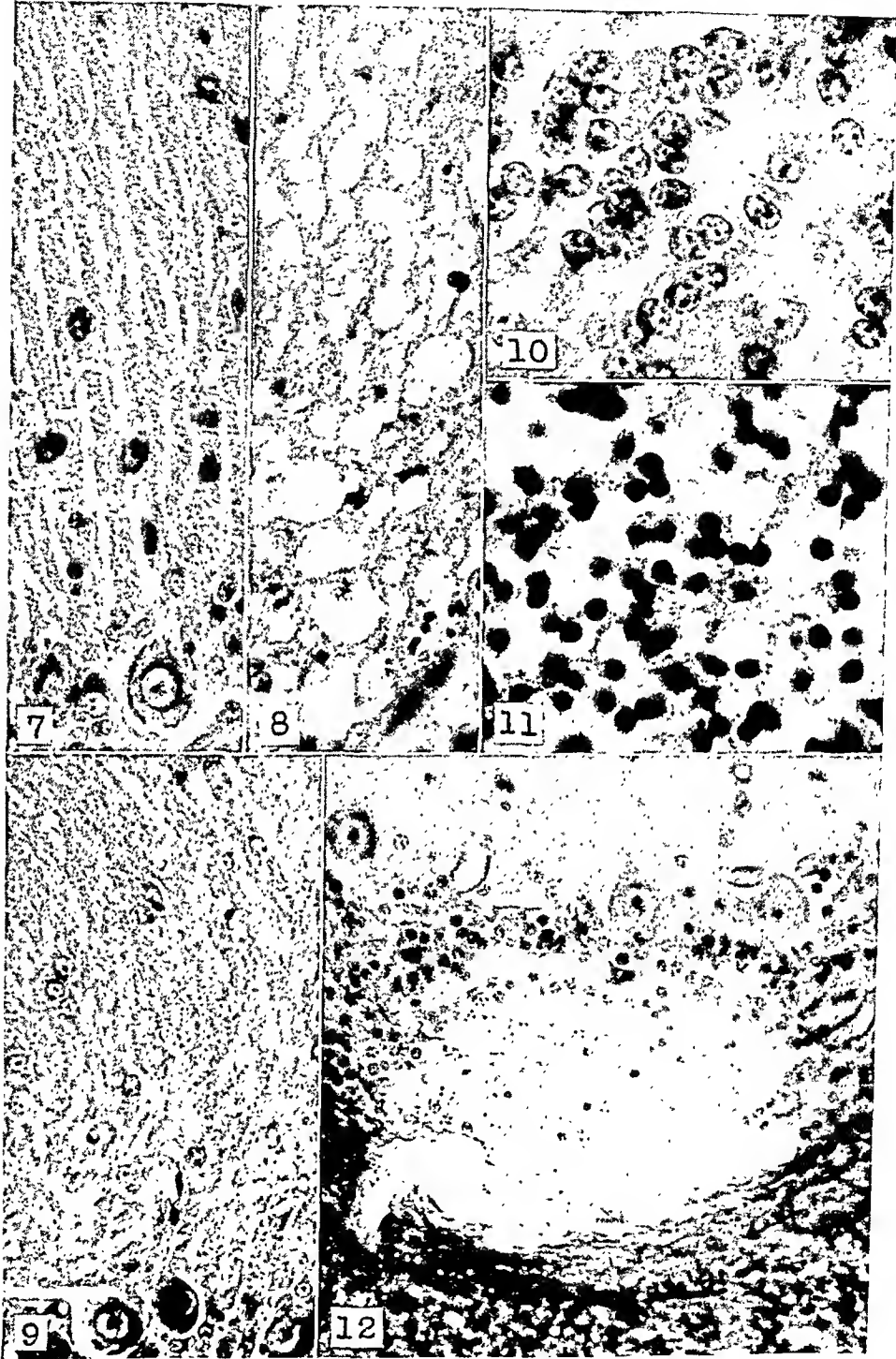
Fig. 2.—Longitudinal section of the cerebellum of a vitamin E-deficient chick.

Fig. 3.—Longitudinal section of the cerebellum of a vitamin A-deficient chick showing the characteristic pinpoint lesions.

Fig. 4.—Longitudinal section of the cerebellum of a vitamin E-deficient chick showing the characteristic A-deficiency lesions.

Fig. 5.—Brain of vitamin E-deficient chick showing a hemorrhage at the base of the cerebellum.

Fig. 6.—Pinpoint lesions in the brain stem of a vitamin A-deficient chick under higher magnification.



FIGURES 7 TO 12
(See legends on opposite page)

contrasts strikingly with the A-deficient brains, all of which except two were free from E-deficiency lesions. In the two excepted (lot 6) E deficiency had been experimentally superimposed on A deficiency.

DETAILED HISTOLOGIC COMPARISONS

It is proposed now to describe in detail the lesions in various parts of the brains of the experimental chicks so that they may be readily compared. The lesions are so distinctive that a carefully prepared series of photomicrographs is presented to show the contrast between similar areas of normal, vitamin E-deficient and vitamin A-deficient brains.

Vitamin E-Deficient Brains.—In E-deficient brains degeneration is usually confined to the cerebellum, although similar changes may occur more rarely in the cerebrum. The general histologic picture seen in the cerebellum may be outlined briefly as follows:

- (a) Outer molecular layer: disorganization of the dendrites of Purkinje cells, producing first a reticulated appearance and finally complete disorganization (fig. 8).
- (b) Pyknosis and degeneration of the cells of the Purkinje layer (figs. 8 and 20).
- (c) Pyknosis and degeneration of cells of the inner granular layer (fig. 11).
- (d) Disorganization of the fibers of the medullary layer of the convolutions (fig. 14).
- (e) Extensive hemorrhage and hyaline thromboses occurring in all layers of the cerebellum. These are seen in the degenerate areas even at low magnifications (fig. 2).

The picture of degeneration described is typical of ischemic necrosis, and it is usually sufficient to enable the observer to diagnose nutritional encephalomalacia caused by vitamin E deficiency. In the present study another detail has come to light which makes the distinction even more certain. In the fibers of the medulla of the cerebellum where extensive disorganization has occurred there are often found areas which have a peculiar reticular appearance. At higher magnifications, however, this is seen to be caused by blister-like swellings on the individual nerve fibers. These vary greatly in size and frequency of occurrence, some fibers

EXPLANATION OF FIGURES 7 TO 12

Fig. 7.—Outer molecular layer of the cerebellum of a normal chick.

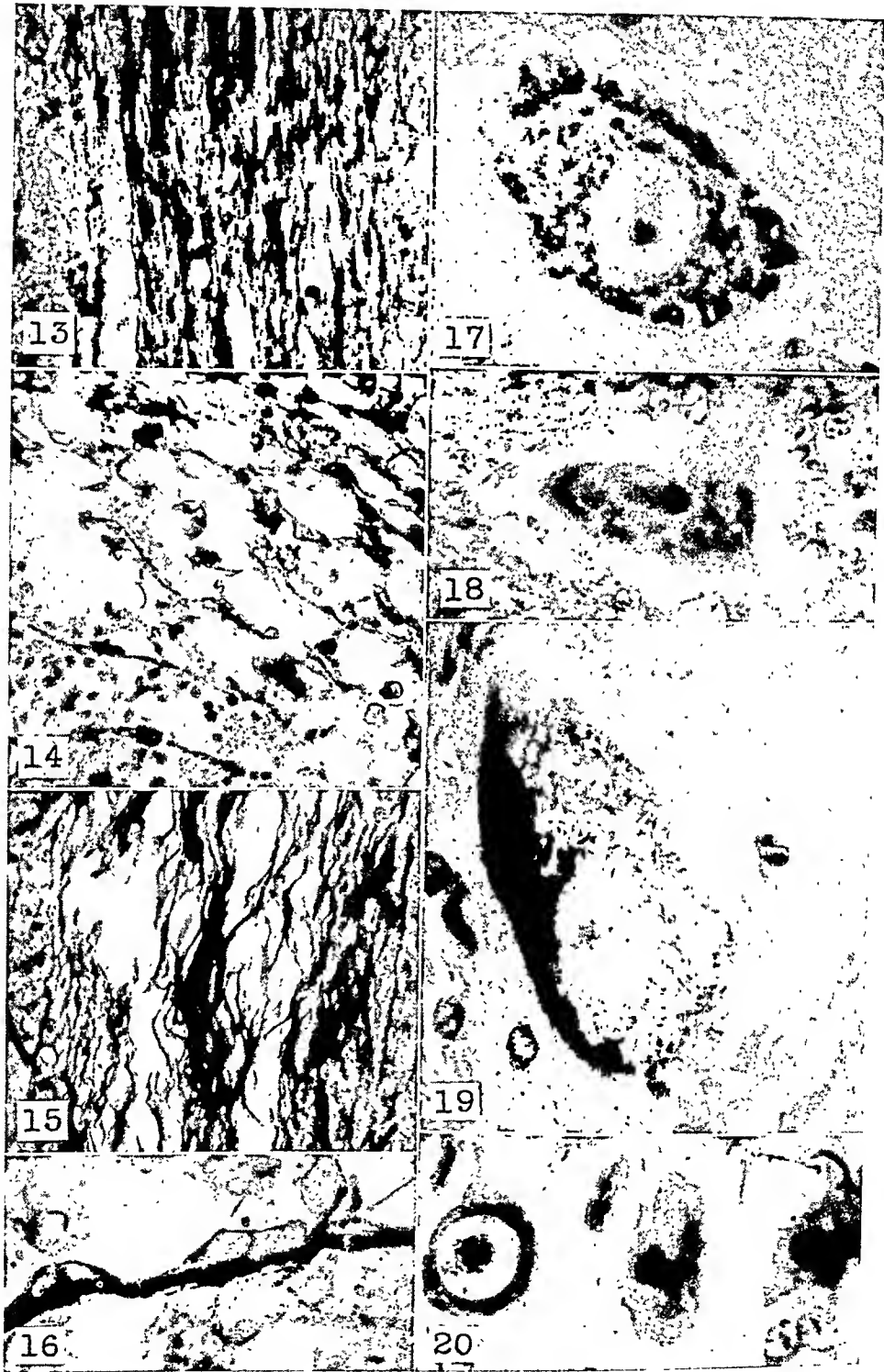
Fig. 8.—Outer molecular layer of the cerebellum of a vitamin E-deficient chick. The disintegration of dendrites produces a typical reticular appearance.

Fig. 9.—Outer molecular layer of the cerebellum of a vitamin A-deficient chick. The dendrites show little abnormality.

Fig. 10.—Cells of the inner granular layer of a normal brain, showing clear vesicular nuclei.

Fig. 11.—Pyknosis of nuclei of cells of the inner granular layer of the cerebellum of a vitamin E-deficient chick.

Fig. 12.—Degenerate area in the inner granular layer of the cerebellum of a vitamin A-deficient chick. Nuclei and cytoplasm have lost their chromaticity, and there is some loss of chromaticity in Purkinje cells at the right.



FIGURES 13 TO 20
(See legends on opposite page)

having few and others appearing almost moniliform (figs. 14 and 16). Examined under the oil immersion objective the swellings appear to be formed by the lifting of a delicate sheath (possibly an axolemma) from the surface of the nerve fiber. This could conceivably be caused by fluid accumulating within the fiber where the vesicles occur. Frequently a series of irregular holes appear to be present in the swollen sheath as if it had burst under pressure. The occurrence of this sheath and of the blister-like vesicles has been found uniformly in E-deficient brains, both those from birds receiving the iron-treated ration and those from birds receiving the purified diet. They were also found in material prepared several years ago. It may be remarked that these structures are present in material fixed and stained with a variety of technics and hence cannot be regarded as artefacts.

The degenerative changes of the cerebrum were similar to those of the cerebellum as regards both the gross and the minute changes. In the brain stem shrinkage and pyknosis of neurons occurred with considerable frequency, but many specimens also showed pinpoint lesions in both the brain stem and the optic chiasma similar to those of A-deficient birds.

Vitamin A-Deficient Brains.—The general appearance of the pinpoint lesions found in brains of vitamin A-deficient chicks is shown in figure 6. Here the lesions appear as conspicuous lighter areas against the more uniform dark background of apparently normal nerve fibers. Under higher magnifications the centers of these areas usually are seen to contain faintly staining nerve cells, and, as shown in figure 6, the process of disintegration appears to spread outward in all directions from these centers. Often the margins of these areas are marked off by an intense staining reaction. It also appears that some of the larger nerve fibers pass through such areas seemingly unaffected by the process of degeneration. Since the cerebellum of vitamin E-deficient chicks is so strikingly affected, as shown in the foregoing section, a detailed description of the cerebellum of the A-deficient chick is given now for purposes of comparison:

(a) Outer molecular layer: As shown in figure 9, the outer dendritic layer of the cerebellum is not greatly affected and by comparison with the normal control (fig. 7) appears to be nearly or quite normal.

(b) Purkinje cell layer: No clear evidence of abnormality was discovered in this layer in any of the A-deficient material (fig. 9), although in the lesion

EXPLANATION OF FIGURES 13 TO 20

Fig. 13.—Fibers of the medulla of the cerebellum of a normal chick.

Fig. 14.—Disintegration of fibers of the medulla of the cerebellum of a vitamin E-deficient chick. Note the blister-like swellings on the fibers.

Fig. 15.—Medulla of the cerebellum of a vitamin A-deficient chick. Note the slight rarefaction of fibers.

Fig. 16.—Single fiber from the medulla of a vitamin E-deficient chick. Note the blister-like swellings and apparent holes in the raised membrane.

Fig. 17.—Neuron from a normal brain showing typical Nissl substance and large vesicular nucleus.

Fig. 18.—Neuron from the brain stem of a vitamin E-deficient chick. Note the general shrinkage and pyknosis affecting Nissl bodies and nucleus.

Fig. 19.—Neuron from the brain stem of a vitamin A-deficient chick. The neuron borders on a pinpoint lesion and shows characteristic disintegration of the nerve fiber and of the Nissl substance in the portion of the cell within the lesion.

Fig. 20.—Pyknosis and degeneration of Purkinje cells of the cerebellum of a vitamin E-deficient chick. Compare with figure 18.

shown in figure 12 there is a slight reduction of stainability, suggesting an ultimate loss of chromaticity.

(c) Inner granular layer: In rare cases conspicuous lesions occurred in this area (fig. 12). They resembled the pinpoint areas of rarefaction seen in the brain stem. The cells had lost their chromaticity, and their nuclei had become clear and vesicular.

(d) Medulla: The nerve fibers show little indication of disorganization beyond the fact that there is a definite thinning out in some regions. Iron-hematoxylin preparations show that this is probably due to demyelination of the fibers (fig. 15 vs. fig. 13).

Degeneration of Neurons in the Brain Stem: Degeneration of the larger neurons in the brain stem is the most striking reaction in A-deficient chicks, and the process of disintegration is probably similar to that which takes place in all other nerve cells that are affected (fig. 19). The cell shown in the illustration lies at the edge of an area of rarefaction, and in it the Nissl substance is undergoing degeneration where it lies in contact with the rarefied area. The Nissl granules have shrunk into thin threads and flakes which stain lightly even though they are still coarse and heavily stainable on the other side of the cell. The nucleus remains clear and vesicular but its chromatin shows a distinct loss of chromaticity as compared with the normal cell (fig. 17).

Degenerate Areas in the Cerebrum and the Optic Chiasma: Rarefied areas indicative of cellular degeneration occurred in the cerebrum in a few A-deficient birds but their location was variable. In the optic chiasma, on the other hand, lesions occurred consistently throughout the whole group (fig. 3), and it may be suspected, therefore, that some visual derangement may have been present in the A-deficient chicks.

COMPARISON OF OTHER STUDIES ON VITAMIN A AND VITAMIN E DEFICIENCY

It seems desirable to compare the histologic observations described herein with those reported by other workers who have investigated the effects of vitamin A deficiency on the nervous system. Many of these studies have been concerned with degenerative changes occurring in nerve fibers. Steenbock, Nelson and Hart⁹ and Hughes, Lienhardt and Aubel¹⁰ noted myelin degeneration in the spinal cord and in peripheral nerves in vitamin A deficiency in swine. They also noted similar changes in cattle and fowl. Mellanby reported degenerative changes occurring in the spinal cord of A-deficient dogs¹¹ and degeneration of neurons in the gasserian ganglion.¹² Seifried,¹³ working with chicks which had been given an A-deficient diet fifteen to eighteen days after hatching, encountered incoordination and nervous disorders at four months. Lesions were found in the brain and the spinal cord involving degeneration of ganglion cells of the cerebral cortex, the dentate nucleus of the medulla and ganglion cells of the anterior horn of the spinal

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cord. Demyelination was noted in the spinal cord and in peripheral nerves. Zimmerman¹⁴ observed vacuolation, swelling and chromatolysis in Nissl preparations of nerve cells of the spinal cords of rats fed an A-deficient diet. Radhahrishna Rao¹⁵ studied neurons of the gasserian ganglion in rats, rabbits and fowls and found disorganization of the Nissl substance of these cells.

In all instances cited in the foregoing paragraph in which nerve cells have been studied, the degenerative changes observed appear similar to those encountered in the present study. By contrast, however, other workers have failed to note nervous disorders or to find lesions in nerve tissues of animals fed vitamin A-deficient diets. This is true of Suzman, Müller and Ungley,¹⁶ working with adult dogs, of Grinker and Kandel,¹⁷ working with monkeys, and of Swank and Davenport,¹⁸ observing rats and guinea pigs. An explanation of the discrepancy between the observations of these authors and those cited in the foregoing paragraph may possibly lie in the fact that the most striking evidences of deficiency diseases are produced in young animals whereas much of this work giving negative results was done with older animals.

Another study of direct interest is that of Duncan,¹⁹ who compared the nerves of rats fed diets low in vitamins A and E with those of normal animals. He was unable to find consistent differences. It is to be noted, however, that the study was confined to nerve fibers and also that no details are given concerning the diets of the experimental animals.

More recently Wolbach and Bessey²⁰ and Mellanby²¹ studied the rate of growth of nerve structures in relation to that of associated skeletal structures. They found that slow growth of skeletal elements accompanied by bone dysplasia causes excessive pressure which causes degeneration of neurons both directly and indirectly. Such an explanation appears to be untenable in the present instance; for, while the skeletal structures of A-deficient chicks grow more slowly than normal, their brains are also much smaller (fig. 1 vs. fig. 3), and there is no evidence of distortion of the brain.

COMMENT

The histologic abnormalities described in this paper show clearly that the condition of incoordination occurring in vitamin E-deficient chicks is associated with extensive destruction of the cerebellum supplemented by lesions of the brain stem. In vitamin A deficiency, however, incoordination is undoubtedly brought about by destruction of neurons of the brain stem and possibly also, to some extent, by visual difficulties occasioned by lesions occurring in the optic chiasma. It is also evident that the occurrence of pinpoint lesions is characteristic of vitamin A deficiency since no other type of lesion was found in these

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brains except two in which a condition of vitamin E deficiency had been deliberately superimposed. Even in these two only the smallest beginnings of lesions of E deficiency were found. In brains of E-deficient birds, on the other hand, degenerative changes of the cerebellum and other parts of the brain were nearly always complicated by areas of rarefaction similar to those seen in the brains of A-deficient birds. It must be concluded, therefore, that some degree of vitamin A deficiency had been established and that this was due to spontaneous changes in the E-deficient food, as maintained by Patrick and Morgan,⁸ or is proof of a synergistic relation between vitamins A and E.

SUMMARY AND CONCLUSIONS

Young chicks reared on a ration deficient in vitamin A or a ration deficient in vitamin E show typical conditions of motor incoordination. These conditions occurring with the two vitamin deficiencies are difficult to distinguish on the basis of clinical symptoms, but both are associated with destruction of nerve cells in characteristic regions of the brain. In E-deficient chicks the lesions are found typically in the cerebellum, while in A-deficient chicks the lesions are mostly in the brain stem and the optic chiasma. Other regions of the brain may be affected in both deficiencies but the location of such lesions is variable.

Microscopic study has shown that the brain lesions are clearly diagnostic of vitamin A or vitamin E deficiency and may be distinguished on the following basis.

Vitamin E deficiency:

Gross lesions: General disintegration of the cerebellum, accompanied by greenish yellow discoloration, hemorrhage and edema. Rarely lesions occur in the cerebrum.

Microscopic lesions: Degeneration of neurons in the affected areas, characterized by pyknosis and shrinkage of cell bodies; degeneration of nerve fibers, accompanied by formation of blister-like vesicles on the fibers.

Vitamin A deficiency:

Gross lesions: None.

Microscopic lesions: Pinpoint areas of degeneration in the brain stem, the base of the cerebellum, the optic chiasma and (rarely) in the cerebrum.

Degenerating cells lose their chromaticity but do not undergo shrinkage. Nissl substance breaks up into fine granules and filaments before undergoing complete dissolution.

Although the E-deficient birds were receiving vitamin A supplements at levels which should have given complete protection, the fact that lesions characteristic of A deficiency regularly occurred in their brains suggests that an intimate relationship exists between the two vitamins. It is clear that either the relation is a synergistic one or vitamin E has a protective role in relation to vitamin A.

ADAMANTINOMA IN THE TIBIA

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EPITHELIAL neoplasms mimicking the structure of the budding enamel body and called adamantinoma or ameloblastoma usually occur in the jaw.¹ Craniopharyngioma, a neoplasm with similar or identical structure, is occasionally observed at the base of the skull.² A case of adamantinoma of the ulna³ and several of adamantinoma of the tibia have been described. Recently Hebbel⁴ tabulated 15 cases of adamantinoma of the tibia, to which 2 cases reported by Dockerty and Meyerding⁵ and 1 by Cagnoli⁶ have been added since. Because of the problems which these growths present as to cellular origin, diagnosis and treatment, the following case is reported in some detail.

A 24 year old white woman was admitted to the University of Oklahoma Hospitals, Oklahoma City, Nov. 14, 1945, with the complaint of a painful swelling of the middle and lower portions of the right leg. She stated that in August 1943 she had fallen and struck her leg against a step. The leg was painful for several weeks, but she was able to carry on her usual activities. The painful area, over the crest of the tibia, about 4 inches (10 cm.) proximal to the ankle joint, gradually decreased. In February 1944 the pain returned, necessitating the use of a crutch in walking. She discarded the crutch early in April. About three weeks later she slipped on a marble floor and "broke her leg." The fracture was reduced without anesthesia and a boot cast applied. The cast was removed in six weeks. When bearing of weight was resumed two weeks later, painful swelling of the leg followed. The swelling increased, and the pain persisted. In October 1945 a roentgenographic examination disclosed a "bone tumor," and amputation was advised.

On admission she appeared well developed, with evidence of some recent loss of weight. Systemic examination gave essentially negative results. There was a

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5. Dockerty, M. B., and Meyerding, H. W.: *J. A. M. A.* **119**:932, 1942.

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fusiform enlargement of the middle of the right leg, extending to within 3 cm. of the ankle joint. The mass was nonfluctuant and had a bony consistency. The overlying skin was tense, shiny and nonadherent, and there was no increase in local heat. The mass was apparently continuous with the tibia. There was moderate swelling of soft tissues over the fibula and the adjacent regions, with no apparent involvement of the fibula. The pulse in the dorsalis pedis artery was feeble, and the capillary circulation in the beds of the toe nails was poor.

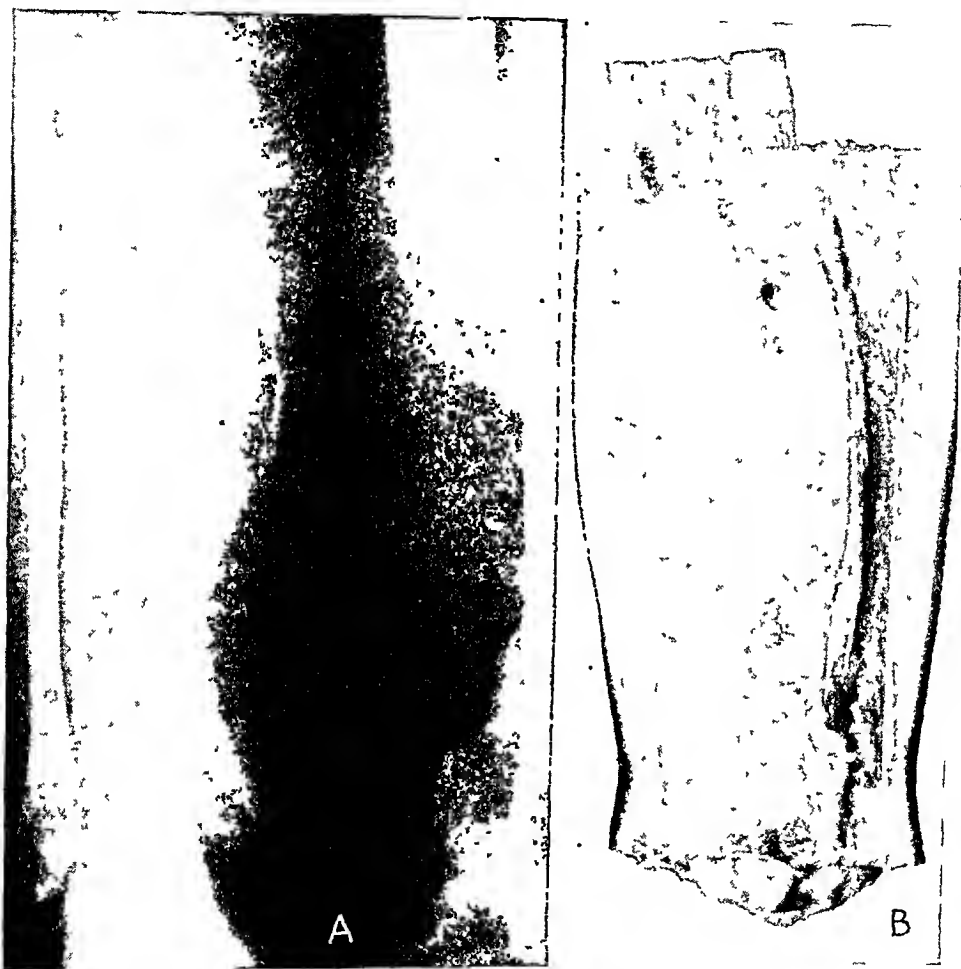


Fig. 1.—*A*, roentgenogram of the tibia, showing an osteolytic lesion destroying the shaft for a length of about 12 cm. *B*, cut surface of the leg, exposing the area of neoplastic involvement.

Roentgenograms disclosed an osteolytic mass which had destroyed approximately 12 cm. of the distal part of the shaft of the tibia (fig. 1*A*). There was expansion of the bony cortex, with only a thin shell remaining. The fibula and the ankle joint appeared intact. Roentgenograms of the chest disclosed no involvement of osseous or soft tissues.

The urine was neutral, cloudy, with a specific gravity of 1.030; it revealed no albumin and no sugar. The red blood cell count was 3,980,000; the hemoglobin content was 12 Gm; the white blood cell count was 14,800, with polymorphonuclears

79, eosinophils 2, monocytes 1 and lymphocytes 18 per cent. The Mazzini and Kolmer-Wassermann tests of the blood were both strongly positive on two occasions.

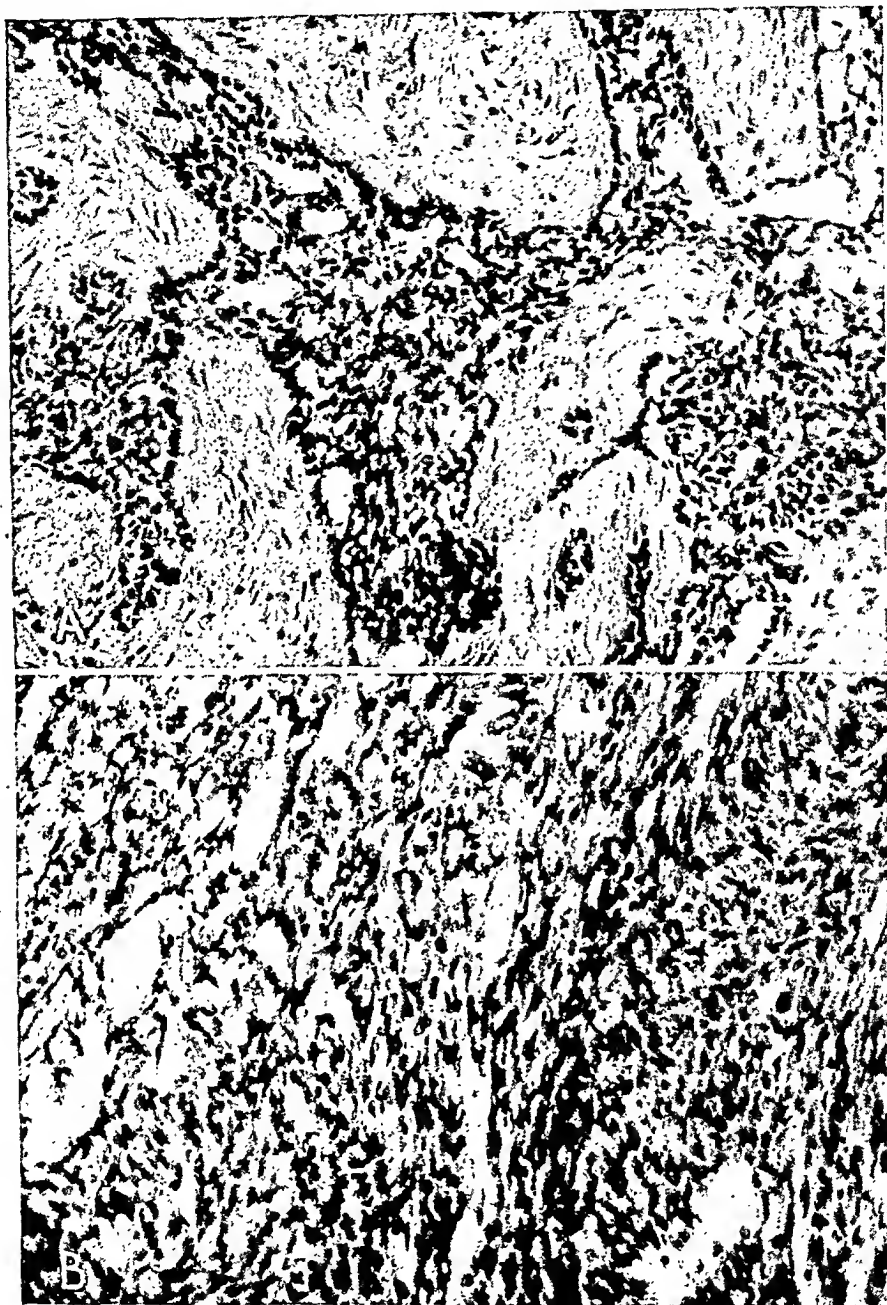


Fig. 2.—Microscopic appearance of the growth. *A*, shows the palisade arrangement of the peripheral cells. In the center of each cell nest there is a stream or whorl-like arrangement of the cells and a lacelike pattern with small and large spaces between cells. $\times 225$.

The clinical impression was: osteolytic bone tumor. On Nov. 17, 1945 the leg was amputated at the junction of the middle and distal thirds of the right thigh, with primary closure of the stump. The postoperative course was uneventful, and

the patient was discharged nineteen days later. When she returned on April 4, 1946, the stump was well healed and she was in good health.

On the extremity, amputated 12 cm. proximal to the knee joint, there were no erosions or discolorations of the skin. A subcutaneous hard fusiform prominence, 8 by 6 by 2 cm., apparently continuous with the tibia was felt commencing about 9 cm. above the level of the inner malleolus. On the cut surfaces (fig. 1 *B*) gray-white neoplastic tissue filled the marrow cavity of the tibia and replaced the compact layer, extending through it into the soft tissues. A convex line of demarcation separated the growth from the soft tissues laterally and a similar wavy convex line bordered it proximally and distally. The growth involved an area about 15 cm. long and 8 cm. wide.

Microscopic preparations stained with hematoxylin and eosin, representing many parts of the growth, disclosed sheets and nests of epithelial cells in a loose fibrous connective tissue stroma. In places the cells formed streams or whorls, with a palisade arrangement of the peripheral cells (fig. 2 *A*). They had deeply stained large round or oval vesicular nuclei with a hardly discernible cytoplasm, which faded into a delicate fibrillar substance. A number of cells were seen in a state of division. In some of the cell nests the cells were in a lacelike pattern, with small or large spaces between them (fig. 2 *B*). Dense hyalinizing fibrous connective tissue bordered the growth and was invaded by cell nests. Apparently compressed striated muscle bundles were seen outside the capsule of the growth.

COMMENT

The cellular origin of adamantinoma of the tibia has intrigued all observers since Fischer⁷ reported the first case in 1913. Epithelial cell rests are frequently found about the jaws and about the base of the skull in the region of the sella turcica. Adamantinoma most frequently occurs at these sites. Epithelial cell rests have not actually been observed in the tibia, yet the microscopic structure of these growths arising in the tibia is similar to, if not identical with, that of adamantinoma or of craniopharyngioma. The skin over the growth remains uninvolved. The growth extends from inside the bone outward. These observations suggest that adamantinoma of the tibia, like that occurring elsewhere, is probably derived from an embryonal rest of the ectoderm. The environment of adamantinoma is similar at all sites where the tumor occurs: that is to say, the growth is in close proximity to periosteum or to osseous tissue. The simple range of variation of the cellular pattern of adamantinoma suggests that the tumor is of more recent genetic origin than the anlage tumors of the salivary glands. In the latter the stroma is a part of the neoplasm whereas in adamantinoma and craniopharyngioma it is a contribution of the surrounding connective tissue.⁸

Adamantinoma is essentially a benign neoplasm with locally invasive properties. It grows slowly and usually shows no areas of necrosis or

7. Fischer, B.: *Ztschr. f. Path.* 12:422, 1913.

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hemorrhage. Cells in a state of division, however, are frequent in many parts of the growth. No distant metastases have been observed in cases of adamantinoma of the tibia,⁹ although a case of Dr. Henry F. Hunt, of the George F. Geisinger Memorial Hospital, Danville, Pa., as yet not reported, appears to be the first instance in which the tumor has metastasized. The growth may be recognized by its characteristic clinical behavior and roentgenographic appearance. Early amputation is the treatment of choice.

SUMMARY

An epithelial anlage tumor—adamantinoma—of the tibia of a 24 year old white woman is reported. This is the nineteenth recorded instance of such a growth of the tibia.

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PARACOLON BACILLUS ENDOCARDITIS OF THE PULMONIC VALVE SECONDARY TO INFECTED POLYCYSTIC KIDNEYS

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THIS is the report of a case of endocarditis of the pulmonic valve due to a paracolon bacillus and occurring secondarily to abscesses of polycystic kidneys. No report of a case of paracolon bacillus endocarditis has been found in the literature.

Bacterial endocarditis of the pulmonic valve is relatively rare, and solitary infection of this valve extremely so.¹ In most of the cases the lesion has occurred on a valve with a congenital or an acquired malformation.² Few cases of solitary bacterial endocarditis occurring on a previously undamaged pulmonic valve have been reported.³ In a survey of 7,000 autopsies performed at the New York Hospital and the Cornell University Medical College since 1917 no other cases of bacterial endocarditis of a previously undamaged pulmonic valve have been found.

Organisms of the Bacteriaceae group seldom are primary in bacterial endocarditis. Schilling⁴ listed reports of endocarditis due to *Escherichia coli*,⁵ *Escherichia acidilactici*,⁶ *Eberthella typhosa* (or *typhi*) and *Aerobacter aerogenes*. Wells observed a case in which "Bacillus paratyphosus B" (*Salmonella paratyphi* B) was the causative organism.⁷ Such endocarditis is often secondary to infections of the abdominal or pelvic cavities.

The paracolon bacillus was first recognized as a distinct organism by Mair in 1906.⁸ He found it associated with acute infections of the urinary tract. However, general acceptance of it as a separate organ-

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7. Wells, H. G.: *Arch. Path.* **23**:270, 1937.

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ism has been recent. It is a gram-negative, seldom motile, rod-shaped organism with colonies resembling those of *Bacillus coli* in form but dirty white with a suggestion of pink in the center on McConkey's agar medium. It is regarded as an intermediate between *B. coli* and the paratyphoid group and differs culturally from *B. coli* in that it ferments lactose slowly, poorly or not at all. It differs biochemically from the paratyphoid group in that it gives a positive indole reaction, and it also does not agglutinate with paratyphoid agglutinating serums. A similar, hemolytic, organism was isolated by Dudgeon from acute infective processes involving the gastrointestinal and urinary tracts. He mentioned two deaths, one of an infant with multiple septic foci in the renal cortices and the other of a patient with "acute pyelocystitis." In both the paracolon bacillus was apparently the infective agent.⁹

REPORT OF A CASE

A 40 year old white man, a waiter, stated that a sharp pain developed in the left lower quadrant of the abdomen rather suddenly in January 1946, with hematuria, urgency and frequency. A diagnosis of polycystic kidneys was made, and nephrocystotomy was performed on the left side at that time. The wound became secondarily infected and drained for some time, then gradually healed.

In May 1946, while in a nursing home, he had a sudden onset of severe cramping spasmodic periumbilical pain which lasted a few hours and recurred the next day with chills, fever and frank hematuria. After four days he was admitted to the New York Hospital. His pulse rate was 90, respiratory rate 25, and blood pressure 150 systolic and 90 diastolic. One observer noted slight enlargement of the heart and a basal systolic murmur. Studies indicated an obstruction of the common bile duct, and at operation, May 23, a chronically inflamed gallbladder and a stone of the common bile duct were removed. Two blood cultures made before operation were sterile. A perinephric abscess was first noted on the sixth day after operation, and two days later it was incised and drained. Culture of material taken from this abscess on two occasions revealed only paracolon bacilli. A blood culture made on the day of incision of the abscess showed only paracolon bacilli, as did another made seven days later. For two weeks after operation the patient had a rapid pulse (rate, 95 to 120) but no fever. Ten days postoperatively a fistula developed from his perinephric abscess, and a biliary fistula as well, with profuse drainage. From his fourteenth postoperative day on he had daily elevations of temperature reaching approximately 38 C. (100.4 F.). On his eighteenth postoperative day a fecal fistula appeared in the lower part of the abdominal wound.

He had received 50,000 units of penicillin intramuscularly every three hours during the first nineteen days after the operation; thereafter he received 4 Gm. of sulfadiazine daily by mouth. Uremia, which was moderate on admission, gradually increased, and edema appeared. His anemia increased despite many transfusions of whole blood. Acidosis developed and rapidly became more severe. His temperature never rose above 38.4 C. (101.1 F.) despite his overwhelming infection. At the time of his death on the fifty-fourth day following cholecystectomy the blood urea nitrogen was 136 mg. per hundred cubic centimeters. No changes of cardiac

9. Dudgeon, L. S., and Pulvertaft, R. J. V.: *J. Hyg.* **26**:285, 1927. Dudgeon, L. S.; Wordley, E., and Bawtree, I.: *ibid.* **21**:168, 1922.

status were noted during his illness, and no petechiae were seen in the skin or the mucous membranes.

Autopsy (two hours after death).—The body was cachectic, weighing only 45 Kg. There was a slight yellow tint to the skin and the scleras. The biliary fistula drained a main radical of the hepatic duct. The fecal fistula extended in over the upper pole of the left kidney, between it and the spleen, and was apparently continuous with the peritoneal cavity at this point; many adhesions were noted here. The kidneys were huge and occupied much of the abdomen. The right kidney weighed 1,380 and the left 1,020 Gm. The capsules were from 3 to 5 cm. in diameter. Some of the cysts contained blood and some thick, cloudy fluid but most contained thick, foul-smelling purulent material. Little functioning renal tissue remained. The pelves were stretched, but the ureteropelvic junctions and the ureters were not remarkable.

The heart weighed 400 Gm. and was globular in shape with predominance of the left ventricle. The walls of the left and right ventricles were 24 and 7 mm. thick,



Fig. 1.—Endocarditis of the pulmonic valve and adjacent endocardium due to the paracolon bacillus. Note the large vegetation on the left anterior cusp. Approximately the actual size is shown.

respectively. The endocardium was thin and delicate except in the region of the pulmonic valve (fig. 1). Here there were numerous firm, flattened, yellow vegetations in the endocardium of the pulmonary conus and the pulmonary artery. The cusps of the pulmonic valve were almost entirely destroyed by similar vegetations. One huge vegetation (4 by 3 by 0.5 cm.) was attached to the left anterior cusp and flapped in the conus like an accessory valve. It was composed of firm, yellow granulation tissue and was not ulcerated. Many of the smaller vegetations were ulcerated. The bases of the three cusps were delicate, and there was no fusion of the commissures. The valve ring was 7 cm. in circumference. The other valves were thin and delicate, and their chordae tendineae were thin and not fused. The coronary arteries had little sclerosis.

The lungs weighed 600 Gm., and the vessels were prominent. The spleen weighed 550 Gm. and was dark red and firm. The liver weighed 2,450 Gm. and was red-brown and firm. The hepatic and common bile ducts were not obstructed. The other viscera were not remarkable. The brain was not examined.

Bacteriologic Examination.—Postmortem cultures were made of the heart's blood, the right lower lobe of the lung, and pus from the cysts of the right and left kidneys. A piece of the large vegetation of the pulmonic valve was put in 70 per cent alcohol for about five minutes and then placed in a sterile Petri dish. Later it was crushed and cultured. A gram-negative rod-shaped bacillus that was culturally and biochemically a paracolon bacillus was grown from each of these sites. From the heart, the lung and the vegetation the paracolon bacillus was the sole organism grown, but from the kidneys *E. coli*, *Clostridium welchii* and a nonhemolytic streptococcus, gamma type, were also grown. The requirements met in its isolation were that it gave a positive indole reaction; it produced no fermentation in lactose during ten days' observation; it produced acid and gas in dextrose, maltose, mannite, xylose, arabinose, salicin and sorbitol. It gave no reac-

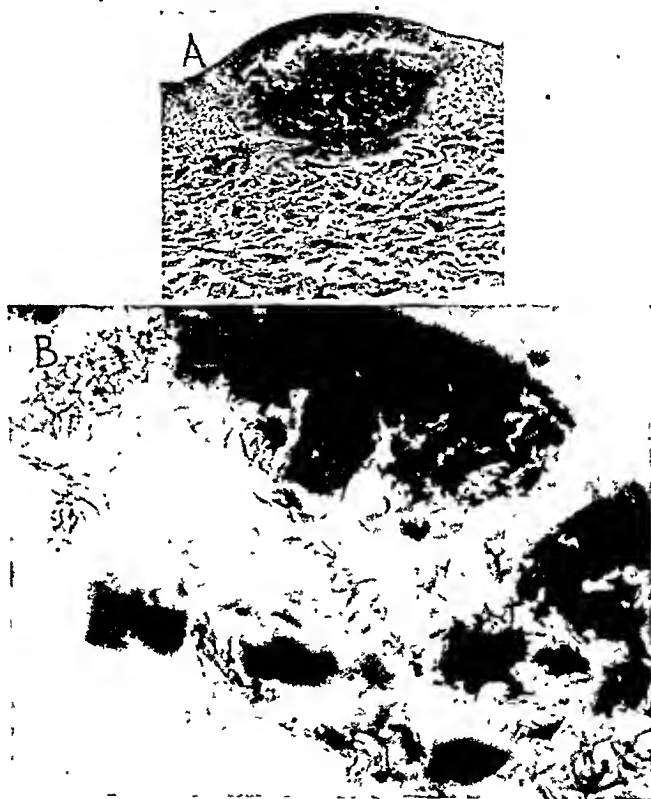


Fig. 2.—*A*, acute lesion of the pulmonary conus, magnified approximately 90 times; hematoxylin and eosin stain. Lesions larger than this were ulcerated. Here the myocardium is infiltrated to some extent by inflammatory cells.

B, paracolon bacilli in the large vegetation, magnified approximately 700 times; Giemsa stain.

tion in saccharose, inosite, urea, lead acetate and gelatin. It produced acid slowly in litmus milk. No attempt was made to classify the paracolon bacillus into a subgroup.

Microscopic Examination.—Sections of the pulmonic valve and the pulmonary conus and artery stained with hematoxylin and eosin revealed beneath the endocardium a fibroblastic proliferation which in places showed mucoid degeneration. The vegetations seen in the gross were composed of focal thickenings due to the fibroblastic proliferation and cellular infiltration accompanying a fibrinopurulent process that was causing destruction of the endocardium (fig. 2 *A*). The infiltrating

cells were mostly polymorphonuclear leukocytes. Small rod-shaped bacteria were present in large numbers in the vegetations examined. With a modified Brown's stain for bacteria (Gram) these stained a brilliant red. No other bacteria were seen in an extensive examination (fig. 2B). The myocardium of the pulmonary conus was infiltrated by leukocytes in areas directly beneath the vegetations. However, the media of the pulmonary artery was infiltrated by both leukocytes and fibroblasts for a considerable area.

Sections of the lungs revealed marked congestion of the capillaries and some atelectasis, as well as thickening of the walls of the arterioles and small arteries.

Hematoxylin and eosin-stained sections of both kidneys revealed the capsules moderately thickened and almost the entire parenchyma replaced by cysts and fibrous tissue. The cysts contained blood, precipitated protein, or purulent material consisting of hemolyzed red blood cells and many polymorphonuclear leukocytes. The cysts were lined with cuboidal or flat epithelium. There were many areas both of coagulation necrosis and of mucinous degeneration. Hemosiderin-laden phagocytes were numerous in the fibrosed areas. A small area of comparatively uninvolved cortex was noted, but even here one saw atrophy and necrosis of some tubules and congestion of the glomeruli. In other areas the cortex was markedly compressed by neighboring cysts.

Similarly stained sections of the parathyroid glands revealed small, relatively inactive glands. The pancreas was fibrotic. The liver, the spleen and other organs were not remarkable.

Anatomic Diagnosis.—Infected polycystic kidneys (culture reveals paracolon bacilli, *E. coli* and *Cl. welchii*); fistula from the upper pole of the left kidney to the skin of the flank; uremia (blood urea nitrogen, 66 mg. per hundred cubic centimeters); slight hypertrophy of the heart; bacterial endocarditis of the pulmonic valve (culture reveals paracolon bacilli). (The leaflets of the pulmonic valve are almost completely destroyed and are covered with vegetations, many of them small and one large. There are also many small vegetations in the endocardium of the neighboring pulmonary conus and artery. Small rod-shaped gram-negative bacteria are present in large numbers in these lesions.) There were chronic cholecystitis, cholelithiasis and choledocholithiasis, with operative removal of gall-bladder and stones; biliary fistula to the right upper quadrant of the abdomen; fecal fistula from the cecum to the right lower quadrant of the abdomen; cachexia (height, 174 cm.; weight 45 Kg.); marked arteriosclerosis of the small arteries and arterioles of the lungs and slight sclerosis of the aorta and coronary arteries.

COMMENT

That bacterial endocarditis may be a consequence of septicemia which, in turn, is a consequence of an acute or chronic infectious process occurring elsewhere in the body is generally accepted. It is also known that the causative agent of bacterial endocarditis may be any one of a large number of bacteria. The mechanism of production of such endocarditis is still not agreed on, and this case gives support to no particular hypothesis. It is generally considered that a previously damaged valve is more apt to be invaded by bacteria than an undamaged one. The pulmonic valve is the least frequently affected by bacterial endocarditis of all the cardiac valves. In only 1 per cent or less of several recorded series of cases of this disease has the pulmonic valve alone been the seat of a bacterial lesion as it was in this case.¹

Careful gross and microscopic examination of the heart in this case failed to reveal any evidence of valvular lesions preceding the current one. The leaflets of the aortic, mitral and tricuspid valves were thin and delicate. There was no vascularization of the cusps, no fusion at the commissures, no thickening of the chordae tendineae. The bases of the cusps of the pulmonic valve were delicate, and there was no fusion at the commissures. The usual three cusps were present. Microscopically, there was no vascularization of the remnants of the cusps and no fibroblastic proliferation away from the acutely infected areas. Therefore, it is probable that there was no preexisting damage of this valve.

As no one has previously identified the paracolon bacillus with lesions other than those of the kidney, it is necessary to rule out the possibility that there was in the lesion of the valve a coexisting or a preexisting organism. Cultures of the perinephric abscess and of the blood made during life produced only pure growths of the paracolon bacillus. Cultures of pus taken from cysts of the right and the left kidney showed growths of the paracolon bacillus, *E. coli*, *Cl. welchii* and a nonhemolytic streptococcus, gamma type. Careful search of Giemsa-stained sections of the vegetations revealed only small rod-shaped bacteria. Gram stains showed only gram-negative organisms in the vegetations. Some investigators have suggested that the paracolon bacillus results when a strain of *E. coli* is modified in the body of the host and, to support this contention, have presented evidence of changing and variable cultures of material taken from the same subject over a period of time.¹⁰ However, it seems reasonable to assume that in this case the endocarditis was initiated and maintained by a paracolon bacillus infection alone. There is no evidence here to support the theory that an organism has been modified within the host.

Chemotherapy was ineffective in controlling this infection. The lesions in the pulmonary conus and artery were acute, involving the myocardium and the media. The extreme debility of this patient, due to chronic renal insufficiency and acute obstruction of the biliary tract, may have lowered his resistance to what is ordinarily a weakly pathogenic organism.

SUMMARY

A case of massive vegetative endocarditis of the pulmonic valve due to the paracolon bacillus is reported. The other cardiac valves were normal. The valvular lesion and the septicemia were secondary to infected polycystic kidneys. No report of a similar case has been found in the literature.

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HODGKIN'S SARCOMA OF UTERUS

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THE STUDY of Hodgkin's disease has received a new impetus from the recent comprehensive investigations of Jackson and Parker,¹ who introduced the concept of three clearly defined clinical and pathologic entities of the disease, namely, Hodgkin's paraganuloma, granuloma and sarcoma. The case to be reported falls into the third group of this classification and is noteworthy for the unusual involvement of the uterus and the extremely advanced age of the patient.

REPORT OF A CASE

M. A. S., an 81 year old white woman, was admitted to the Taunton State Hospital and her condition diagnosed as senile psychosis. The father and one brother had died of cancer. The patient had never been seriously ill. She had been married for sixty-two years and had three children. The mental illness began two years prior to admission with irritability, silly actions, such as cutting up clothes and giving away household objects, and ideas that she was persecuted by her daughter, with whom she was living. On admission she was found to be a senile patient of 105 pounds (47.5 Kg.) and of 59 inches (148 cm.) in height. The blood pressure was 165 systolic and 70 diastolic. Physical, neurologic and vaginal examinations gave negative results. The hemoglobin content was 10.6 Gm.; the red blood cell count, 3,859,000; the white blood cell count, 6,500, with a differential count of 70 per cent polymorphonuclear cells, 8 per cent stab cells, 1 per cent eosinophil cells and 21 per cent lymphocytes. The blood was of type A. The Kahn and Hinton tests were negative. The urine was acid, with a specific gravity of 1.024, and there were no significant chemical or microscopic changes. A stool was negative for enteric pathogens. After a nine months' observation period, during which she had been tidy and cooperative, the patient was released to go home for a trial visit. However, a few months later she became unmanageable again and was returned to the hospital. The subsequent course was generally unchanged until her death, which took place with the signs of heart failure about two years after her admission.

Autopsy (thirteen hours after death).—The body was that of an 83 year old white emaciated woman measuring 59 inches in length and weighing about 90 pounds (about 41 Kg.). There was extreme wasting of adipose and muscular tissues. The sacral region and buttocks showed a large decubital ulcer. The finger nails and the lips were cyanotic. The right pupil was larger than the left.

On opening the peritoneal cavity a markedly enlarged uterus was found projecting domelike from the lesser pelvis above the symphysis. It measured 7 by 10 by 15 cm. and corresponded in size to that of about four months' gestation. The upper surface was covered with smooth serosa; the lower portion of the

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1. Jackson, H., Jr., and Parker, F., Jr.: *New England J. Med.* **230**:1, 1944; **231**:35 and 639, 1944.

corpus was continuous with fused infiltrated lymph nodes lining the wall of the lesser pelvis and extending upward around the aorta. These masses of tumor tissue surrounded all structures, especially vessels of the lesser pelvis, without causing obstruction except of the right ureter slightly above its juncture with the bladder. The cervix was not involved and was normal except for old lacerations of the external orifice. The uterine cavity was compressed and displaced to the anterior and left side. The endometrium was thin and tan colored, with some small hemorrhages in the fundus. The myometrium of the posterior wall was completely replaced by white tumor tissue with some necrotic and hemorrhagic areas in the center. There was also diffuse infiltration of the anterior and the lateral wall of the myometrium. The adnexa attached to the lateral aspects of the upper, serosa-covered portion of the uterus were atrophic and free of tumor tissue. The vulva and vagina were atrophic.

The lymphoid system showed, in addition to the infiltrated pelvic and aortic nodes, some enlarged mesenteric nodes. On section these nodes were white and firm, with occasional areas of necrosis. The spleen weighed 90 Gm. and showed a smooth capsule and soft hemorrhagic pulp.

The heart weighed 200 Gm. and was flabby. The myocardium showed brown atrophy. The aortic valves and the coronary arteries were sclerosed and partly calcified. The aorta and the major arteries showed a moderate degree of arteriosclerosis. The lungs were congested, the lower lobes edematous and the upper lobes moderately emphysematous, with old apical scars.

The intestines were not remarkable except for the rectum, which was surrounded by tumor tissue at the level of the pelvic floor. The pancreas weighed 90 Gm.; the liver, 990 Gm. Both organs, as well as the gallbladder and the bile ducts, were without gross changes.

The kidneys showed marked contraction, the left weighing 50 Gm. and the right 30 Gm. The capsules stripped with moderate difficulty leaving finely granular surfaces. On section the right kidney showed evidence of hydronephrosis, marked dilatation of the pelvis and calices and atrophy of the parenchyma, which in places measured as little as 4 mm. in thickness. The left kidney showed a normal pelvis but narrowing of the cortex and medulla. The right ureter was compressed by tumor tissue near the bladder and above this point was dilated up to 8 mm. in diameter. The bladder was surrounded at the fundus by tumor tissue, which, however, did not infiltrate its wall.

The adrenal glands weighed 5 Gm. and showed some autolytic changes. The pituitary gland was of normal size and shape. There was marked osteoporosis of ribs and skull. The central nervous system showed adherence of the dura to the calvarium, fibrosis of the arachnoid and marked arteriosclerosis. The brain after fixation in solution of formaldehyde weighed 1,360 Gm. and showed moderate cortical atrophy of the frontal and parietal lobes. Coronal sections revealed a cavity measuring 5 mm. in diameter in the right lentiform nucleus and a small area of softening in the white matter of the right frontal lobe.

Microscopic Examination.—Microscopic sections of the uterine fundus (fig.) showed mainly tumor tissue, which in some areas was seen infiltrating between smooth muscle fibers. The tumor cells, which were arranged in sheets with partly scanty, partly dense stroma, were markedly pleomorphic, with hyperchromatic nuclei and cytoplasm varying in staining reaction from basophilic to acidophilic. The most conspicuous cells were giant cells (fig. 2) measuring from 15 to 35 microns; many were multinucleated, and many showed large round, lobulated or indented nuclei. The latter consisted of mostly dark-staining, irregular chrom-

atin; some contained prominent nucleoli. Some cells showed mitotic figures. The cytoplasm of these giant cells varied also in staining reaction from basophilic to slate colored to acidophilic. Blood vessels were abundant and consisted of single layers of endothelial cells. There were large areas of hemorrhage and necrosis. One

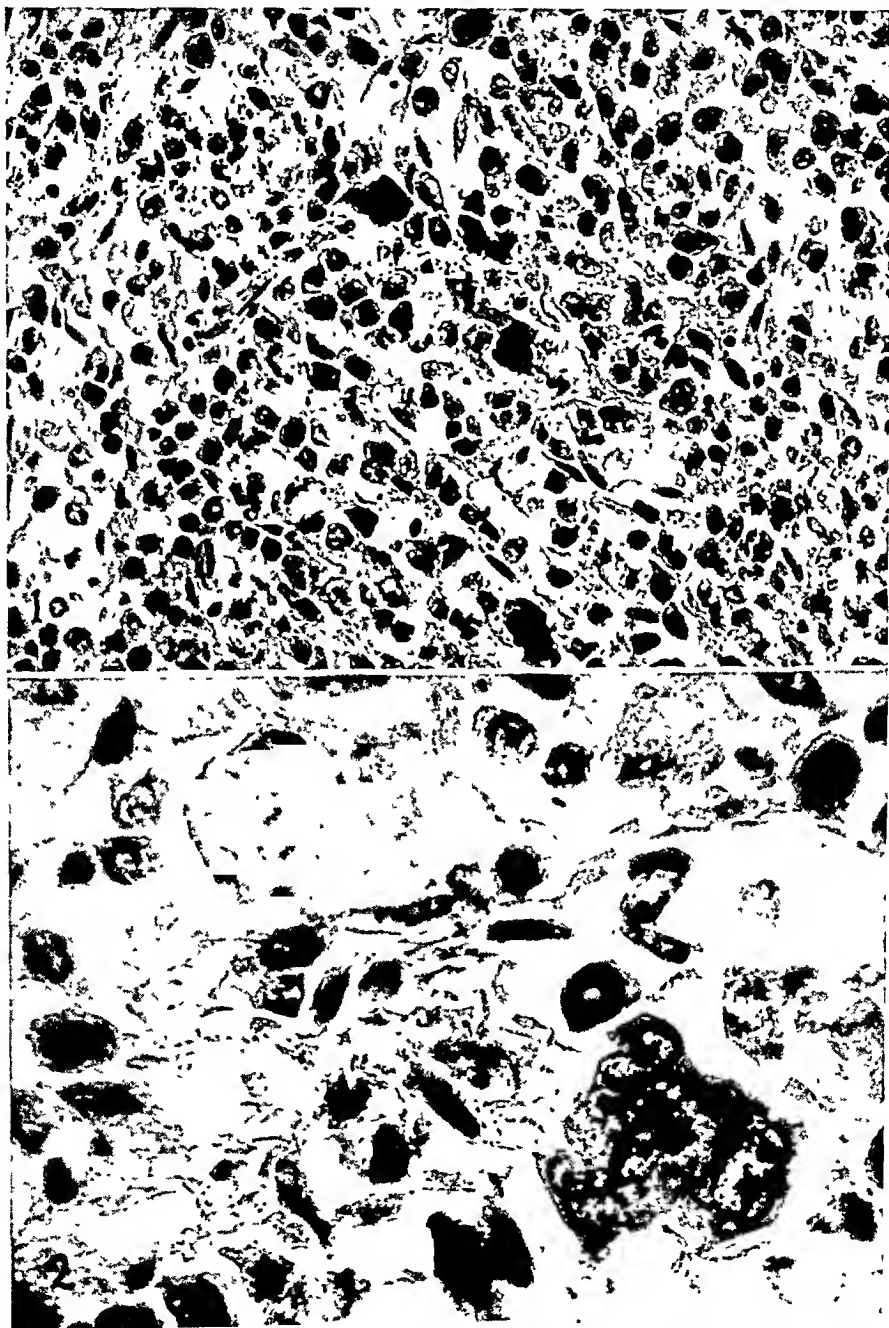


Fig. 1.—Typical field of uterine tumor showing pleomorphism and Reed-Sternberg cells. Hematoxylin and phloxine; $\times 250$.

Fig. 2.—Two large cells of Reed-Sternberg type. Hematoxylin and phloxine; $\times 900$.

TABLE 1.—*Summary of Cases Reported Since 1912*

Author	Age	Organs Involved	Uterus		Other Data
			Macroscopic	Microscopic	
Ullströmer ⁴ (1933)	39	Lymphatics, spleen, liver, mesentery, uterus	15 cm. greatest dimension; diffuse infiltration; endometrium necrotic	Hodgkin's sarcoma, infiltrating between muscles; vessels thrombosed	Duration 1½ years
Lewinski ³ (1930)	43	Lymphatics, spleen, liver, bone marrow, lungs, kidneys, bladder, urethra, skin, peritoneum, vagina, uterus, tubes	Nodes in serosa; infiltration and necrosis of cervix resembling cancer	Hodgkin's granuloma	Cachexia; white blood cell count from 2,020 to 28,000
Scenes ² (1939)	35	Lymphatics, tonsils, pharynx, intestine, spleen, pleura, bladder, ovaries, tubes, uterus	9 by 5 by 1.6 cm.; intramural white tumor with indistinct demarcation; white nodes in serosa	Hodgkin's granuloma	Anemia; white blood cell count 10,800; dilated heart
Waltherd, K. M.: Ztschr. f. d. ges. Neurol. u. Psychiat. 97:1, 1925	35	Spinal canal (extradural), uterus	White nodule, 1.1 by 1.1 by 0.7 cm.; border not sharp	Incomplete autopsy
Schlagenhauser, F.: Arch. f. Gynäk. 95:7, 1912	68	Lymphatics, spleen, pancreas, mesosalpinx, mesovary, plexus uterovaginalis	Massive growth in plexus uterovaginalis	Incomplete description
Jessup, W. E. D.: Proc. New York Path. Soc. 12:3, 1912	43	Lymphatics, spleen, skin, tubes, one ovary; simultaneous tuberculosis	12 by 9 by 6 cm.; diffuse infiltration, fibrosis	Hodgkin's granuloma	
Present author (1946)	83	Lymphatics, uterus	15 cm. greatest dimension; diffuse infiltration, neurosis	Hodgkin's sarcoma	Duration less than 2 years; no clinical signs

region showed endometrial glands. Silver impregnations revealed a rather dense network of reticulum fibers enmeshing tumor cells.

Sections of the cervix showed diffuse infiltration with tumor tissue identical in type with that described in the fundus uteri. There were areas of well preserved myometrium.

The aorta showed marked intimal deposits of lipids and calcium. Adjacent to the adventitia there were remnants of lymphoid tissue infiltrated diffusely by tumor cells identical with those described.

COMMENT

The histologic features of marked pleomorphism, of giant cells resembling those of Reed and Sternberg and of reticulum can be considered characteristic of Hodgkin's sarcoma, which in this case was restricted to the abdominal lymphatic system and uterus. The preferential involvement of the lymphoid system in Hodgkin's disease makes it plausible to assume that the process originated in the pelvic nodes and from there invaded the uterus, the posterior wall of which was the main site of infiltration.

TABLE 2.—*Hodgkin's Sarcoma in Old Persons*

Author	Cases	Age
Krueger and Meyer ⁶	69	Oldest 74 ♀ 84 ♂
Uddströmer, M.: Acta tuberc. Scand. 1934, supp. 1, p. 1	536	5 months to 77
Williams, J. E., and Oliver, T. M.: Texas State J. Med. 32: 486, 1936	11	12 ♀ Negro to 77 ♂ white
Goldman, L. B.: J. A. M. A. 114: 1611, 1940.....	212	6 to 76
Bruger, R. E., and Lehman, E. P.: Arch. Surg. 43: 839, 1941	54	4 to 79
Baker, C., and Mann, W. N.: Guy's Hosp. Rep. 89: 83, 1939	65	7 to 67
Haden, R. L., and Burns, J. T.: Cleveland Clin. Quart. 9: 144, 1942	47	3½ to 67
Stewart, F. W., and Doan, C. H.: Ann. Surg. 93: 141, 1931	24	16 (1 ♂ 1 ♀) to 57
Jackson and Parker ¹		
Paragranuloma	38	8th decade 1 patient
Granuloma	237	9th decade 1 patient
Sarcoma	51	8th decade 7 patients
Sailer, S.: Am. J. Clin. Path. 6: 241, 1936.....	74	8th decade 2 ♂ 1 ♀
Mills, E. S., and Pritchard, J. E.: Canad. M. A. J. 33: 50, 1935	20	8th decade 1 patient

An opinion on the incidence of uterine involvement in Hodgkin's disease can be gained from Jackson and Parker's large series comprising a total of 326 cases of all types. On detailed analysis these authors reported that of 59 cases of Hodgkin's granuloma with autopsy the uterus was involved in 2 and that among 27 cases of Hodgkin's sarcoma there was 1 with uterine involvement—an incidence of 3.4 per cent and 3.7 per cent, respectively. On surveying the more accessible literature of the past forty years, reports on 6 more cases were found as compiled in table 1. In all these cases the involvement of the female sex organs represents only one localization of a widespread process which may invade all portions of the genital tract as in Scenes' ²

2. Scenes, A.: Ztschr. f. Geburtsh. u. Gynäk. 96:121, 1929.

case or parts of it as in Lewinski's³ case or the uterus selectively as in Uddströmer's⁴ and the present case. On the other hand, the ovaries may be affected selectively and the uterus spared as in 1 of the 2 cases recently reported by Heller and Palin.⁵ These authors classified their case as an instance of Hodgkin's sarcoma and demonstrated tumor cells within veins suggesting a spread through the blood circulation. It is interesting to note the close resemblance between Uddströmer's and the present case in the histologic appearance of the tumors, as well as in the diffuse infiltration of the myometrium resulting in uterine enlargement of identical proportion, and in duration of the disease.

Another unusual aspect of the present case is the advanced age of the patient, who died at 83 years. Data compiled from the literature in table 2 show only 1 patient older than the present one, a man aged 84 years, whose case was reported by Krueger and Meyer,⁶ and only 1 among Jackson and Parker's 237 patients with Hodgkin's granuloma, listed as being in the ninth decade of life. This rarity of Hodgkin's disease in the very old depends on the fewer survivors in the higher age brackets, as shown by Minot and Isaacs'⁷ finding that the decreasing incidence of lymphoblastoma with advancing age roughly parallels the age distribution of the living population. The present case, however, appears to illustrate well that Hodgkin's disease may not even spare women at an age of more than 80 years.

SUMMARY

A case has been described in which Hodgkin's sarcoma involved the uterus of a senile psychotic person 83 years of age.

3. Lewinski, H.: *Zentralbl. f. Gynäk.* **54**:2824, 1930.
4. Uddströmer, M.: *Virchows Arch. f. path. Anat.* **289**:486, 1933.
5. Heller, E. L., and Palin, W.: *Arch. Path.* **41**:282, 1946.
6. Krueger, F. J., and Meyer, O. O.: *J. Lab. & Clin. Med.* **21**:682, 1936.
7. Minot, G. R., and Isaacs, R.: *J. A. M. A.* **86**:1185, 1926.

Laboratory Methods and Technical Notes

A PEROXIDASE REACTION IN PARAFFIN SECTIONS

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FOLLOWING the introduction of the Romanowsky stains, Maximow showed that the delicate neutrophilic granules, which are so distinct in smears and imprint preparations, are indistinct and uncertain in tissue sections.¹ In the best of paraffin sections most neutrophils show merely a "granular appearance" rather than separate individual granules.

Up to the present there is only one fairly reliable way of demonstrating individual neutrophil granules in sections, namely, the using of one of the peroxidase reactions in frozen sections. Sato and Shoji,² Graham,³ Jacoby⁴ and others have applied these reactions with and without gelatin embedding.

In the course of studies made on sections of bone marrow, a need arose to show the neutrophilic granules in paraffin-embedded hemopoietic tissues. Since it is known that the peroxidase reaction is completely lost after paraffin infiltration, and since, on the other hand, it is known that the stained granules are stable once the peroxidase reaction has been applied, it occurred to us that the reaction might be used before the start of the embedding process. In this way, it was thought, one might imitate the "en bloc" staining used in the carmine staining of whole pig embryos or the Golgi methods employed for the central nervous system.

Pencils of the femoral marrow of rats and guinea pigs were obtained by the method of Mayer and Ruzicka.⁵ With this technic the medullary tissue is made accessible to the fixative within five minutes after the thigh is opened, and decalcification is avoided throughout.

We used a procedure in which Graham's peroxidase reaction is modified by the neutralization of the formaldehyde fixative and followed this with a routine sequence of embedding and sectioning.

PROCEDURE

Part 1: Peroxidase reaction.

1. Fixation—Twenty-four hours.

95 per cent ethyl alcohol.....	90 cc.
37 per cent solution of formaldehyde U. S. P....	10 cc. pH approx. 7.0
0.1 normal sodium hydroxide.....	1 cc.

2. Wash in running water ten minutes or allow to stand in water one hour.

From the Lederle Laboratories Division, American Cyanamid Company.

1. Maximow, A.: Arch. f. mikr. Anat. **76**:47 and 55, 1910.
2. Sato, A., and Shoji, K. S.: J. Lab. & Clin. Med. **13**:1058, 1929.
3. Graham, G. S.: J. M. Research **35**:231, 1916.
4. Jacoby, F.: J. Physiol. **103**:22P, 1944.
5. Mayer, E., and Ruzicka, A. Q.: Anat. Rec. **93**:213, 1945.

3. Peroxidase reaction—Twenty-four hours.

Alpha naphthol.....	1 Gm.
40 per cent ethyl alcohol.....	100 cc. prepared fresh
Superoxol	0.2 cc.

4. Rinse in running water ten minutes or after several rinses, allow to stand in water one hour.

5. Pyronine stain—Three to twenty-four hours.

Pyronine	0.1 Gm.
40 per cent ethyl alcohol.....	96 cc.
Aniline oil.....	4 cc.

Part 2: Paraffin embedding process.

6. 80 per cent alcohol.....	1 hr.
95 per cent alcohol.....	2 hr.
Absolute alcohol 1.....	1 hr.
Absolute alcohol 2.....	1 hr.
Cedarwood oil.....	Overnight ⁶
Xylene 1.....	20 min.
Xylene 2.....	20 min.
Paraffin 1.....	1½ hr.
Paraffin 2.....	1½ hr.

7. Embed, block and cut at 4 microns.

Part 3: Counterstains (optional).

In addition to the peroxidase reaction, counterstains may be used. The eosinophilic and basophilic granules assume their usual distinctive staining, while the neutrophilic granules remain as individual red dots.

Nuclear stains, e. g., hematoxylin, do not interfere with the visibility of the granules.

Cytoplasmic stains, e. g., eosin, may be used but should be applied as a light background for the red granules.

Romanowsky stains, e. g., Kingsley's,⁷ may be used satisfactorily, provided the red component is kept relatively light. In our experience the neutralized formaldehyde fixation permits the use of this type of stain, with good differentiation of all elements. Hemoglobin is well preserved.

SPECIAL POINTS OF EMPHASIS

It is important that the fixation (part 1, step 1) is not extended beyond twenty-four hours. We have not been able to obtain satisfactory results after a longer period. This may be due to the possible production of formic acid. Although this factor does not appear to be disturbing in the frozen section-peroxidase method, a longer washing time is required in that technic and might well be applied here (part 1, step 2).

In our experience the alcohol-formaldehyde fixative (part 1, step 1) gives results superior to those obtained with an aqueous formaldehyde fixative, whether neutralized or not.

We attempted to find out at which step of the paraffin embedding process (part 2, step 6) the peroxidase reaction becomes negative. When

6. This is our routine procedure. Cedarwood oil might well be omitted if a gradual transfer from alcohol to xylene is carried out in some other way.

7. Kingsley, D. M.: Stain Technol. 10:127, 1935.

the reaction was applied after the tissue had been treated with absolute alcohol, the granules were less distinct; after treatment with xylene, the reaction was negative.⁸

RESULTS

In the thinner and less cellular areas of the paraffin sections the delicate neutrophilic granules appear as individual red dots in a large number of those cells, a result which cannot be obtained with the Romanowsky stains in paraffin or celloidin⁹ sections. The presence of one or more granules in any immature cell identifies it with the granulocytic series. Thus this peroxidase procedure distinguishes the granulocytic series from the lymphocytic series at the premyelocyte stage.

In sections which are treated with this peroxidase procedure the eosinophilic and basophilic cells can be distinguished from the neutrophilic by the conspicuous and characteristic size and shape of their granules. However, these coarse granules are equally well demonstrated by any of the Romanowsky methods.

When not counterstained, the section may show a slight pink tinge as a background of the red granules, but this does not interfere with the use of a counterstain.

This peroxidase procedure has been applied to sections of the livers and the spleens of rats and guinea pigs, as well as to sections of their bone marrows. The demonstration of the granules of the hemopoietic cells of these sections has been equally satisfactory. The usual morphologic characteristics and staining properties of the tissues are preserved by the fixative used. Thus it has proved possible to combine the peroxidase procedure with the routine stains of such organs without interfering with other studies of these tissues. Leukemic or suppurative infiltrations of any organ can obviously be treated in the same manner. There is no doubt that the combining of a peroxidase reaction with the paraffin technic will facilitate the differentiating of the myeloid from the lymphoid series of cells in tissues.

SUMMARY

A successful presentation of the neutrophilic granules occurring in paraffin sections of the rat's and the guinea pig's marrow, liver and spleen is accomplished by applying a modification of Graham's peroxidase reaction to freshly fixed tissue prior to the dehydrating and embedding processes.

The preservation of structures and the staining properties of the tissues are not affected by the procedure.

The new procedure is considered as a tool for classifying the hemopoietic cells of the tissues.

8. It is self evident that the tissue must be carried back to 40 per cent alcohol before the peroxidase procedure is applied.

9. Celloidin is a concentrated preparation of pyroxylin.

Books Received

THE CULTIVATION OF VIRUSES AND RICKETTSIAE IN THE CHICK EMBRYO. By W. I. B. Beveridge and F. M. Burnet. Medical Research Council, Special Report Series, no. 256. Pp. 92, illustrated. Price 2 shillings. London: His Majesty's Stationery Office, 1946.

This monograph gives a full, well founded account of the history, the methods and the results of chick embryo cultivation of viruses and rickettsias. The text contains six sections, each with complete references. The cultivation of the individual strains of viruses and rickettsias that are definitely associated with human and animal diseases receives detailed consideration. There is a summary of unconfirmed reports of the cultivation of certain viruses, also of negative results. Chick embryo cultivation of bacteria, protozoa and foreign tissue is reviewed. The book will be a great help to all who deal with viruses, rickettsias and other microbes—the investigator, the public health worker, the maker of vaccines, the teacher and the student.

CLINIQUE ET INVESTIGATIONS. By Noël Fiessinger, professor of the Clinique Médicale à l'Hotel-Dieu and member of l'Académie de Médecine. Pp. 831, with 192 illustrations. Price 750 francs. Paris: Masson & Cie, 1946.

This book deals with the possibilities and limitations of laboratory methods of medical investigation. Its 800 pages are divided into six parts, a division more arbitrary than scientific. It demonstrates that in properly selected cases diagnosis is possible only by laboratory investigation; that in others laboratory tests are valuable aids; that in others they confuse the clinician or discredit the physical findings, and that in a small group, despite their intrinsic worth, they may lead to erroneous conclusions. To illustrate the total reliability of laboratory investigations, cases of anemia, leukemia and miliary tuberculosis are selected. To illustrate the accessory value of laboratory investigations, tests of hepatic and renal function are emphasized in connection with the diagnosis of cirrhosis of the liver and of nephritis. Myocardial infarction and peptic ulcers are other diseases the diagnosis of which is shown to be facilitated by the laboratory. To illustrate the confusion produced by misrepresentations of laboratory results, the author points to cases in which renal glycosuria is treated as diabetes mellitus and cases in which orthostatic albuminuria and hepatic azotemia simulate nephritis.

The fifth part emphasizes the importance of accuracy in taking of the history and recording the results of physical examination in cases in which such entities as ruptured peptic ulcer or acute cholecystitis are considered as diagnostic possibilities. Although his thesis is the complete adequacy of bedside diagnosis, the author admits the value of the leukocyte count and that of the roentgenographic demonstration of subdiaphragmatic air. The fact that these laboratory procedures are considered in this chapter demonstrates how arbitrary are the divisions of this book.

The sixth chapter deals with the errors that arise in diagnosis in spite of good clinical and laboratory investigations. However, the errors described were those incident to incomplete or inadequate or even careless investigations.

This somewhat verbose book brings nothing new to the field of clinical or laboratory investigation. In fact, many new methods are ignored. It may, however, be a valuable demonstration of the fallacy of uncritical interpretations of laboratory results. The errors inherent in such interpretations are generously illustrated and should serve to caution the unwary.

LEHRBUCH DER UROLOGIE. By Prof. Dr. Med. J. Minder, ehem Ordinarius für Urologie und Direktor der Urolog. Universitäts-Klinik Budapest, zur Zeit Spezialarzt der Urologie in Zürich. Cloth. Pp. 344, with 62 illustrations. Price 37.50 Swiss francs. Berne, Switzerland: Hans Huber, 1946. Distributed in the United States and Canada by Grune & Stratton, Inc.

This book is intended as a textbook of urology. There are 25 chapters, comprising 315 pages, covering the principal urologic conditions, followed by a synopsis of urology of 27 pages. The opening chapter is a good sketch of the development of urology as a specialty and of its relation to other branches of medicine. It is followed by chapters on urologic symptomatology and renal function tests. The next 22 chapters cover the most important clinical urologic conditions, with special emphasis on renal tuberculosis (2 chapters), renal stones (2 chapters) and prostatic hypertrophy (4 chapters). However, there are many important topics which are entirely skipped in this main portion of the book—for instance, tumors of the testis, prostatic carcinoma, carcinoma and other lesions of the penis, urethral stricture and its sequelae, neurogenic diseases of the bladder and traumatic conditions of the urinary tract. Some of these are mentioned in the 27 page summary, or synopsis, at the end of the book, while several are not mentioned at all. The reviewer has the impression that the book is comprised of a number of lectures on the most important phases of urology, with the synopsis added at the end in an attempt to fill the gaps left. The printing and the format are good. The illustrations are fewer than in most American textbooks.

News and Notes

Examination to Be Given by the American Board of Pathology.—The American Board of Pathology will give an examination in Philadelphia on June 5 and 6. Applications for this examination will not be accepted after May 1. Inquiries should be directed to Dr. Robert A. Moore, secretary, Washington University School of Medicine, St. Louis 10.

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VISCERAL KAPOSI'S DISEASE

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OUR INTEREST in Kaposi's disease was first aroused by a case of sudden death, diagnosed clinically as due to hypertensive heart disease, that one of us (Tedeschi) had the opportunity to study several years ago. Postmortem examination revealed spongy bluish red nodules in the liver, the left kidney and the small bowel. Microscopically, these nodules were found to have a structure similar to that ascribed to cutaneous Kaposi's disease. Since cutaneous manifestations were lacking at the time of the autopsy and since there was no mention of any in the clinical history, the diagnosis of visceral Kaposi's disease was not felt to be justified and the case was filed as one of "systemic angio-reticulosis," the emphasis being placed thus on the most striking pattern in the hepatic, intestinal and renal nodules: a disorderly proliferation of reticulum cells along various lines, with development of vascular channels.

Many years later (1945) we were confronted with an identical structural pattern in a mediastinal mass, again characterized by an intimate new growth of vascular structures and young mesenchymal cells, in a woman dying of myelogenous leukemia. Although in this case, also, the structural characteristics of the mediastinal mass closely resembled those of cutaneous Kaposi's disease, we felt that this diagnosis was not justified, and the mass was classified as "angioreticuloma."

The present report was prompted by a third similar case which was recently brought to our attention: A boy died of intestinal intussusception who, in the complete absence of cutaneous lesions, showed lymph-glandular, splenic, intestinal and hepatic lesions, the structure of which again simulated the patterns of cutaneous Kaposi's disease. At the time this case was being studied some articles had already appeared which discussed the possibility of "visceral Kaposi's disease" in which the process may remain confined to the internal organs or in which

From the Division of Laboratories and the Medical Service of the Framingham Union Hospital.

the visceral manifestations may precede the cutaneous lesions. The diagnosis of "visceral Kaposi's disease" was made for the third case, and this, together with the two previous similar cases, is reported here, with a further discussion of this new aspect of Kaposi's disease.

REVIEW OF LITERATURE

Medical literature contains numerous references to this uncommon condition described by Moritz Kaposi¹ and labeled first as "idiopathic multiple pigmented sarcoma" (1872), then as "sarcoma idiopathicum multiplex hemorrhagicum" (1894) and currently as Kaposi's sarcoma or multiple idiopathic hemorrhagic sarcoma.

Incidence and Localization.—Some hundred cases of the disease recorded in the literature enabled us to make certain well established generalizations: that the incidence is greater in males, only 5.99 per cent of the patients in the 434 cases described up to 1939 having been females (Choisser and Ramsey²); that the prevailing distribution of the disease is among the populations of Eastern Europe and Northern Italy, though no part of the world lacks representation; that the peak of frequency is in the fifth, sixth and seventh decades of life; that the condition occasionally develops in several members of the same family (Greco³), and that patients of the white races predominate over those of the Negro race.

The extremities, the face and the trunk are the most frequent sites of cutaneous localization. Contrary to Kaposi's expectation, secondary involvement of the internal organs is not frequent, occurring only in about 10 per cent of the cases; spontaneous regression of the nodules has been noted many times, and the disease is no longer regarded as invariably fatal.

Among the later developing visceral localizations, the most frequent is the gastrointestinal tract, including all portions from the mouth to the rectum; however, practically all organs may be involved by the process. Diarrhea, gastrointestinal hemorrhage and hemorrhagic effusion into a serous sac have been occasionally recorded in connection with the intestinal and serosal localizations.

Pathologic Aspects.—Among the earliest manifestations of the disease, a swelling of the extremities, usually symmetric in distribution, has been reported and variously interpreted (Stats⁴; Mierzecki⁵; Hansson⁶). Blockage of lymph drainage was accounted for either by

1. Kaposi, M., cited by Kren.⁸

2. Choisser, R. M., and Ramsey, E. M.: *Am. J. Path.* **15**:155, 1939.

3. Greco, cited by Sutton, R. L., and Sutton, R. L., Jr.: *Diseases of the Skin*, St. Louis, C. V. Mosby Company, 1939.

4. Stats, D.: *J. Mt. Sinai Hosp.* **12**:971, 1946.

5. Mierzecki, H.: *Arch. f. Dermat. u. Syph.* **165**:577, 1932.-

6. Hansson, C. J.: *Acta radiol.* **21**:457, 1940.

infiltration of the lymphatic vessels or by involvement of the regional lymph glands (Dalla Favera⁷; Kren⁸; Aegerter and Peale⁹). In some other cases an explanation was found in coexistent dermic sclerotic changes in proximity to the cutaneous nodules; but a large group of cases still remains in which no apparent cause could be found for the condition.

Another early development in the disease is the more or less general lymphadenopathy affecting both superficial and deep lymph nodes and noticeable at a time when the cutaneous lesions are not yet manifest or are so limited in extent or so indefinite in character as to be easily overlooked or misinterpreted. The enlargement of the lymph nodes does not necessarily indicate that they are involved by the process. In some of Dalla Favera's cases⁷ the only detectable change was a thickening of the fibrous connective tissue stroma of the lymph glands. Hyperplasia of reticulum cells was thought by Dörffel¹⁰ to account for the condition. A similar change was described by Greppi and Bettoni,¹¹ while in Goldschlag's cases¹² the pattern was that of a chronic nonspecific inflammatory process. Plasma cell infiltration, the presence of histiocytic phagocytes containing iron pigment and a moderate thickening of the stroma of the lymph glands led to a similar interpretation in the cases of MacKee and Cipollaro.¹³ It is difficult at present to discuss the significance of these changes; one possibility is that they may represent an aspecific inflammatory reaction; the other possibility is that they may represent early stages of the development of the disease process.

Besides the enlargement of the lymph nodes, Stats⁴ has recently called attention to the concomitant enlargement of the spleen. This organ weighed 1,340 Gm. in the case of Usseglio and associates,¹⁴ 1,000 Gm. in a case observed by Symmers¹⁵ and over 700 Gm. in not a few instances recorded (Stats⁴). In some cases the presence of tumor nodules was found to be responsible for the condition (Symmers¹⁵; Paolini¹⁶), but in a considerable number of other cases no

7. Dalla Favera, G. B.: *Arch. f. Dermat. u. Syph.* **109**:387, 1911.

8. Kren, O.: *Sarcoma idiopathicum haemorrhagicum*, in Jadassohn, J.: *Handbuch der Haut- und Geschlechtskrankheiten*, Berlin, Julius Springer, 1933, vol. 12, p. 891.

9. Aegerter, E. E., and Peale, A. R.: *Arch. Path.* **34**:413, 1942.

10. Dörffel, J.: *Arch. Dermat. & Syph.* **26**:608, 1932.

11. Greppi, E., and Bettoni, I.: *Arch. Ist. biochim. ital.* **4**:403, 1932.

12. Goldschlag, F.: *Dermat. Wchnschr.* **100**:204, 1935.

13. MacKee, G. M., and Cipollaro, A. C.: *Am. J. Cancer* **26**:1, 1936.

14. Usseglio, G.; Zambelli, E., and Paolino, W.: *Gior. d. r. Accad. di med. di Torino* **101**:404, 1938.

15. Symmers, D.: *Arch. Path.* **32**:764, 1941.

16. Paolini, R.: *Rassegna internaz. di clin. e terap.* **8**:514, 1927.

evidence of specific splenic involvement could be noticed. In the case of Greppi and Bettoni¹¹ the findings included a "hemolytic spleen" with marked "arterial hyperemia"; in the case of Usseglio and associates¹⁴ the enlargement of the organ appeared to be due mostly to hyperplasia of macrophagic histiocytes, many of which contained red blood cells. While in the case of Stats⁴ it was due to hyperplasia of reticulum cells.

Changes comparable to those in the spleen were occasionally noted in specimens of marrow obtained by sternal aspiration. Active hyperplastic marrow was found by Stats⁴ in 2 cases, and Bertaccini¹⁷ reported an increase of reticuloendothelial cells and monocytes in 2 other cases. In these 2 cases and in 2 others reported by the same author there were concomitant deviations from the normal in the hemogram of the peripheral blood, mainly a marked increase of the monocytes. Monocytosis of a considerable degree was observed also in some of the cases of Dörffel¹⁰ and Bruns.¹⁸ Flarer¹⁹ recognized as many as 60 per cent abnormal monocytes in the peripheral blood, showing a marked shift toward the cells of the lymphomonocytic series. As for the findings in the spleen and in the lymph nodes, the changes in the marrow and in the peripheral blood were regarded by some as evidence of the widespread involvement of the reticuloendothelial system. The conception of the systemic nature of Kaposi's disease and its relation to lymphoblastoma and reticuloendothelioma have been emphasized lately in papers discussing cases in which Kaposi's disease developed concomitantly with mycosis fungoides (Lane and Greenwood²⁰), or with lymphatic leukemia (Cole and Crump²¹), or with Hodgkin's disease or lymphoblastoma (Gilchrist and Ketron²²).

Histologic Observations.—The variable histologic structure of Kaposi's morphologic unit has presented problems the solution of which has baffled many investigators. The 23 different names applied from time to time to the increasing number of cases (MacKee and Cipollaro¹³) are indicative of the lack of agreement as to the point of origin of the growth.

We are indebted to Dörffel¹⁰ for a fine description of the changes throughout the development of Kaposi's nodule. Among the patterns characteristic of the earliest stages, the author stresses a network of blood capillaries surrounded by a thick mantle of young mesenchymal cells embedded in the meshes of a thin interlacing of argentaffin fibers.

17. Bertaccini, G.: *Gior. ital. di dermat. e sif.* **80**:631, 1939.

18. Cited by Bertaccini.¹⁷

19. Flarer, F., cited by Bertaccini.¹⁷

20. Lane, C. G., and Greenwood, A. M.: *Arch. Dermat. & Syph.* **27**:643, 1933.

21. Cole, H. N., and Crump, E. S.: *Arch. Dermat. & Syph.* **38**:283, 1920.

22. Gilchrist, T. A., and Ketron, L. W., cited by Highman, W. J., in discussion on Lane and Greenwood,²⁰ p. 654.

Sinus-like spaces lined by endothelial cells not unlike those found in the splenic tissue are present and are interpreted as the earliest attempt at formation of new blood vessels. Blocks or granules of iron pigment, either free or within macrophagic histiocytes, are noticeable, and their presence is explained by an escape of red blood cells into the tissue through the incompletely closed blood channels.

In a later stage, corresponding grossly to the "bright red nodule," the vascular network is still more striking, with many vessels and cavernous spaces, lined by one or more layers of endothelial cells, spreading in various directions in a fanlike fashion.

If the proliferation of blood vessels prevails in the process, the resulting pattern will be that of angioblastoma. If, instead, the cellular proliferation is predominant, the growth is likely to bear a striking resemblance to round cell or spindle cell sarcoma.

Spontaneous regression of newly formed nodules is explained on the basis of degenerative changes, shown by the cytoplasmic and nuclear vacuolation, the edema and the myxomatous change of the stroma, at times followed by attempted repair in the form of a proliferation of granulation tissue. These changes are not unlikely to be seen in concomitance with thrombotic changes in blood vessels or independent of them.

The conclusion that Dörffel¹⁰ reached is that the cell type involved basically is the pluripotent cell of the reticuloendothelial system which, owing to its various developmental potentialities, is able to give rise to complex and unpredictable structures.

This conclusion has been accepted by a number of investigators (Guccione²³; Flarer¹⁹; Choisser and Ramsey²), and Goldsmith,²⁴ in a recent article, did not hesitate to classify Kaposi's sarcoma as among the diseases of the reticuloendothelial system. The same view was expressed by Puhr,²⁵ who explained the formation of blood vessels in the growth by fusion and subsequent excavation of cells in which he recognized all the functional and morphologic characteristics of the reticuloendothelial cells. Greppi and Bettoni¹¹ reached a similar conclusion in discussing an unusual case of Kaposi's disease with extensive visceral manifestations and concomitant hemolytic splenomegaly. According to them, the involvement of the reticuloendothelial system in their case had manifested itself in the splenic district with an exaggeration of the hemocatheretic function proper to this organ, while elsewhere it had given rise to a neoplastic growth with the characteristics of Kaposi's disease.

23. Guccione, F.: *Arch. ital. di anat. e istol. pat.* 5:1, 1934.

24. Goldsmith, W. N.: *Brit. J. Dermat.* 56:104, 1944.

25. Puhr, L.: *Dermat. Wchnschr.* 91:1815, 1930.

Yet there is no unanimity concerning the origin of the process. Kren⁸ doubted that the cells primarily involved are the reticuloendothelial cells and stated that the only substantial point in favor of this origin is the presence of cells provided with phagocytic power in the newly formed nodules. The unit of growth in Kaposi's disease, according to Symmers,¹⁵ is the fibroblast, a cell which is able to maintain a low capacity of growth over a number of years and which may suddenly assume active proliferation ending with widespread destruction of tissues as in a spindle cell sarcoma.

Etiology.—Although microscopic studies have led to fairly general agreement as to the histologic features necessary to establish the diagnosis, in the present state of knowledge there is little agreement regarding the etiologic explanation of the condition. Some classify Kaposi's disease among the granulomas and others among the neoplasms. The opinion held by the majority in the past and still shared by some at present (Van Cleve and Hellwig²⁶) is that there is an infectious agent of unknown origin attacking the entire vascular and perivascular system with the lesions assuming neoplastic characteristics in predisposed subjects and under certain conditions. Against this are the crucial experiments of Pack²⁷ and Choisser and Ramsey²⁸ who, using the injection method in human subjects, failed to elicit any sort of reaction.

Recalling Hodgkin's disease, for which a recent classification in subgroups has been found to be satisfactory both from the clinical and from the pathologic standpoint, one might also accept for Kaposi's disease the similar subdivisions proposed by MacKee and Cipollaro,¹⁸ namely, inflammatory, granulomatous and neoplastic processes. Among the cardinal features of the inflammatory pattern the authors list engorgement of blood and lymph vessels, extravasation of red blood cells, and perivascular infiltration by round cells, connective tissue cells and plasma cells. Predominant features of the granulomatous pattern are the proliferation of hematic and lymphatic channels accompanied by pronounced hyperplasia of connective tissue cells. In the neoplastic pattern the picture varies according to the elements predominantly involved: If the hyperplastic-dysplastic process affects the blood vessels mainly, the picture will be that of hemangioblastoma; if, instead, the lymphatic vessels prevail in the growth, the picture will be that of lymphangioma or of a mixture of hemangioma and lymphangioma; fibroma, angiofibroma, angiosarcoma or spindle cell sarcoma may result from excessive and disorderly connective tissue cell proliferation.

The hypothesis of the systemic nature of Kaposi's disease has been advocated by many investigators. In discussing 4 cases in which

26. Van Cleve, J. V., and Hellwig, C. A.: *Urol. & Cutan. Rev.* **39**:246, 1935.

27. Pack, G. T.: *J. M. Soc. New Jersey* **34**:603, 1927.

28. Choisser, R. M., and Ramsey, E. M.: *South. M. J.* **33**:392, 1940.

anatomic and functional changes occurring in blood vessels were noticeable far beyond the Kaposi lesions, Santori²⁹ expressed the opinion that underlying the disease was a congenital or an acquired general debility of the vascular system. Bessone¹⁸ arrived at a similar conclusion, and in 4 of his cases he was able to demonstrate capillary fragility as well as anatomic vascular defects. This was also noticed by Bertaccini,¹⁷ who emphasized the abnormal appearance of the blood capillaries, which displayed thickness and hyalinosis of the walls both in proximity to and at a distance from the lesions. Along the same line is the explanation of Leigheb³⁰ in a case in which Kaposi's disease apparently followed an erythema from arsphenamine. Favre and Josserand ascribed Kaposi's disease to systemic angiomatosis without hesitancy.

It was assumed in the past by most students that the extracutaneous lesions in the disease were metastatic. This appeared to be justified by the fact that up to the time of Dörffel's paper¹⁰ no case had been reported in which lesions of the skin were lacking. The literature since 1932 includes the reports of a few cases in which the involvement of the internal organs seems to have preceded the cutaneous manifestations, and even of some cases in which the cutaneous lesions were lacking altogether.

In Pearce and Valker's case³¹ a nodule in the gum was noticeable several months before the skin became involved. Barringer and Dean³² twice noted primary lesions on the glans penis. Van Cleve and Hellwig²⁶ and Goldschlag³³ reported cases in which the inguinal lymph nodes were primarily involved. Paolini¹⁶ had a patient who complained of intestinal symptoms long before the appearance of the cutaneous manifestations. The pharynx and larynx were primarily involved in a case reported by Bilancioni.³⁴ In the patient of Greppi and Bettoni¹¹ the only apparent external manifestation was in the glans penis; later on the inguinal lymph nodes became involved, but no lesions of the skin developed during the year of illness prior to death. At autopsy the psoas muscles were extensively involved, and nodules were found in the lungs. In the case of Stats,⁴ about fourteen months intervened between the onset of the illness, characterized by symmetric pitting edema of both lower extremities, and the appearance of circumscribed nodules in both legs.

Incidental involvement of the heart and pericardial sac, generally with absence of clinical symptoms, has been frequently recorded as

29. Santori, G.: *Gior. ital. di dermat. e sif.* **73**:782, 1932.

30. Leigheb, V.: *Arch. ital. di dermat. e sif.* **11**:461, 1935.

31. Pearce, C. T., and Valker, L. E.: *Ohio State M. J.* **32**:137, 1936.

32. Barringer, B. S., and Dean, A. L., Jr.: *Tr. Am. A. Genito-Urin. Surgeons* **28**:409, 1935.

33. Goldschlag, F.: *Dermat. Wchnschr.* **100**:204, 1935.

34. Bilancioni, G.: *Rassegna internaz. di clin. e terap.* **13**:1135, 1932.

among the visceral localizations of Kaposi's disease (Symmers¹⁵; Dillard and Weidman³⁵; Stats,⁴ cases 2 and 4). A primary and unique cardiac localization is more unusual. Almost simultaneously, in two independent papers, Weller³⁶ and Choisser and Ramsey² described 2 cases (which Stats suspects to be the same) of primary Kaposi's sarcoma of the right atrium of the heart, classified as angioreticuloendothelioma from the morphologic standpoint. Similar cases were described by da Cunha Motta,³⁷ and more recently Aegerter and Peale⁹ have added another case in which the only apparent localization of the disease was in the right atrium of the heart. The identification of these cases as instances of Kaposi's disease was based on the close resemblance of the cardiac findings to those usually seen in association with the cutaneous manifestations of Kaposi's disease. Whether or not the cases deserve to be considered as such is one of the pertinent points of the present study.

ILLUSTRATIVE CASES

CASE 1.—In North Italy a laborer aged 59 years was admitted to St. Anne's Hospital, Ferrara, Jan. 20, 1938. He was moribund and died after a few hours. The only information that could be obtained was that for a number of years he had had high blood pressure, frequent dizzy spells and symptoms of cardiac decompensation which had greatly limited his activities.

Necropsy.—The body was that of an obese, well developed white man who appeared to be somewhat older than the stated age of 59 years. The lips, the mucous membranes of the mouth and the nail beds of the fingers and the toes were deeply cyanotic, but no other significant changes could be detected by external examination of the body.

There was a large heart, of an estimated weight of 450 Gm., with marked hypertrophy of the left ventricle and severe sclerotic changes of the coronary arteries. There was much myocardial fibrosis, and scarring was noticeable in places. Both lungs were congested and edematous, but no areas of consolidation were found. The kidneys weighed 250 Gm. together and were granular in appearance, with atrophy both of cortex and of medulla, and with formation of cysts. Hyalinosis of glomeruli, arteries and arterioles was later observed in the microscopic sections, and the cause of death was attributed to cardiorenal arteriosclerotic disease.

In the course of the autopsy our attention was mainly attracted by the bluish red nodules present in various organs of the abdominal cavity. One of these nodules, 1.8 cm. in diameter, was in the liver. It was firm, elastic in consistency, smooth and glistening on the surface but spongy on the cut section owing to conglomeration of cavities of different sizes. Some of these cavities were empty, while others contained fluid blood. As there was no capsule, nor any formation resembling a capsule, circumscribing the nodule from the surrounding hepatic parenchyma, the limits between the two tissues were not clearly made out, the nodule blending smoothly into the hepatic tissue.

35. Dillard, C. I., and Weidman, F. D.: *Arch. Dermat. & Syph.* **1**:283, 1920.

36. Weller, G. L.: *Ann. Int. Med.* **14**:314, 1940.

37. da Cunha Motta, L.: *An. Fac. de med. da Univ. de São Paulo* (pt. 2) **17**: 627, 1941.

Another nodule the size of a large pea was embedded in the upper pole of the left kidney. Except for the smaller size it did not differ from the nodule in the liver. Approximately half of the nodule was fleshy and hemorrhagic, while the other half was firm, elastic, compact and somewhat variegated in color because of alternating grayish pink and yellow-tinged areas. The renal nodule also was not sharply demarcated from the surrounding tissue, merging at its borders into the renal parenchyma.

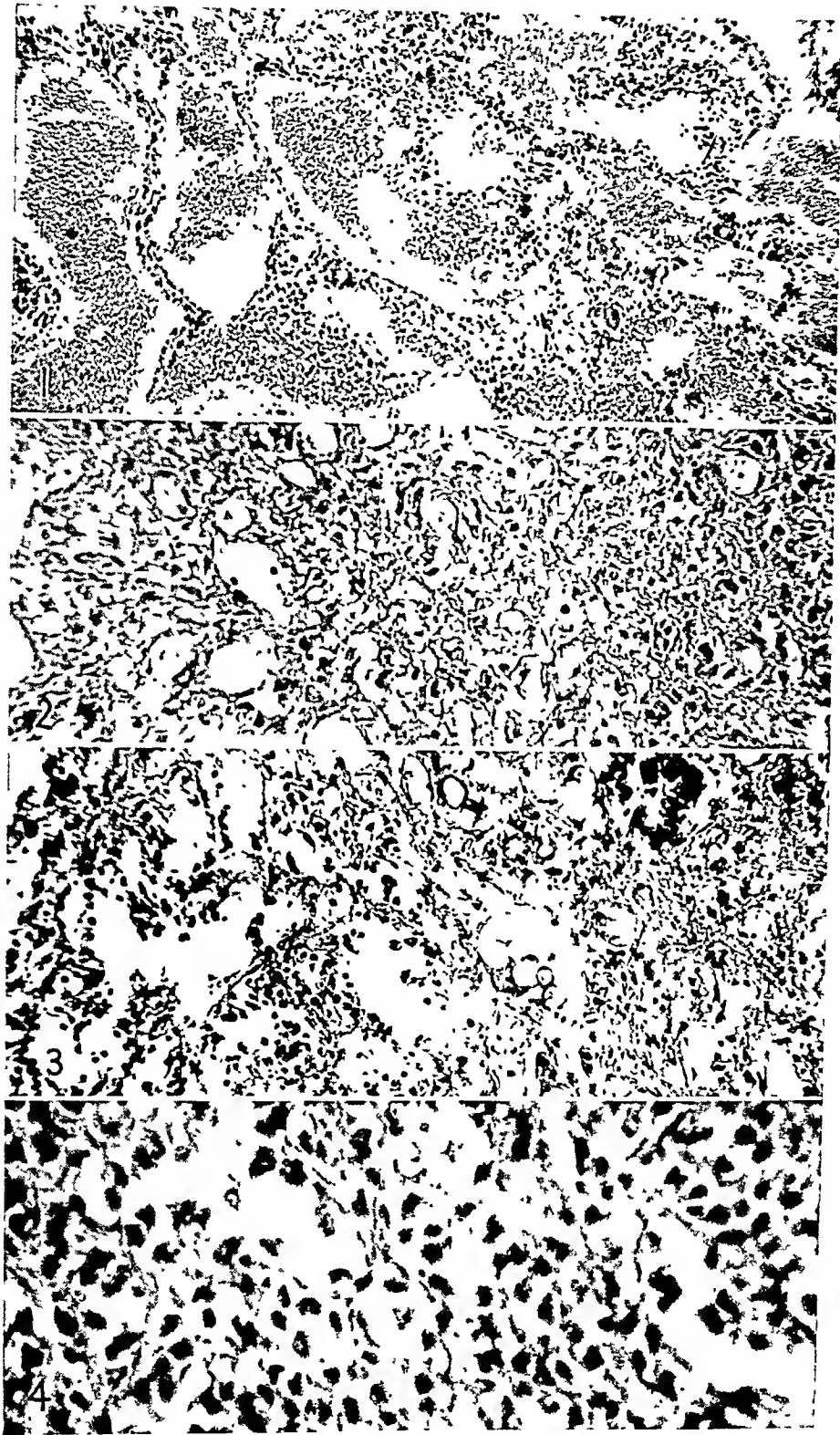
The third nodule was discovered in the small intestine, about 3 feet (91.5 cm.) from the ileocecal valve. Hemispheric in shape and the size of a small cherry, it bulged beneath the intestinal mucosa, which was smooth, glistening and otherwise not remarkable. On the cut section the central portion of the nodule looked like a sponge filled with blood, while at the periphery there was a condensation of tissue gently melting into the intestinal walls.

Microscopic Observations.—The microscopic sections from the nodule in the liver showed it to be composed of an interlacing of young connective tissue fibers, some of which, bending in a circular fashion, resulted in the formation of closed spaces (fig. 1). Some of these spaces were lined by a layer of flattened or fusiform cells regularly alined one after the other, which resembled in all respects the endothelial cells lining the blood and lymph vessels. Red blood cells were contained in some of these spaces, and other red blood cells lay in the surrounding tissue. Scanty connective tissue cells, either sparse or in small groups, could be seen here and there. Some of these cells, with large vesicular nuclei provided with nucleoli and basophilic cytoplasm, had the characteristics of reticulum cells. The majority, however, were spindle shaped, with swollen vesicular nuclei, and displayed all the attributes of young fibroblasts.

The structure of the nodule in the intestine differed somewhat from the structure of the hepatic nodule. The fundamental pattern was still that of thin-walled blood channels embedded in a fibrous connective tissue stroma, hyaline in places (fig. 2). Some of these channels were composed of a single lamella of regularly alined endothelial cells; some others, of an endothelial lamella lying on a thin layer of collagenous tissue. All these channels were greatly dilated, with sinuositities along the walls which here and there appeared surrounded by a scattering of cells mostly fibroblasts and lymphocytes with occasional histiocytes. Well preserved red blood cells were present in the lumens of the blood channels, and more red blood cells, either sparse or in groups, were seen in the meshes of the surrounding fibrous connective tissue stroma. Granules of yellowish brown pigment were seen, mostly within large macrophagic histiocytes, and they were identified as iron granules in the sections stained by Turnbull's blue method.

The limits between the nodule and the surrounding intestinal submucosa appeared to be better defined in the microscopic sections than they had been to the naked eye, because of a palisade of connective tissue fibers in parallel arrangement all around the nodular formation. Yet even beyond this palisade of connective tissue fibers the vascular intestinal pattern appeared distorted. Groups of thin-walled engorged blood vessels stood out conspicuously, and in the absence of inflammatory changes or any other apparent pathologic condition the same disorderly arrangement could be noticed in tracts of small intestine at some distance from the nodule, giving the impression that the vascular defect had more extensive roots than one might have suspected at first.

The structure of the renal nodule was more complicated. It varied from area to area, yet the predominant feature was still that of sinus-like spaces lined by one or more rows of endothelial cells, not unlike the spaces and cells observed in the spleen (fig. 3). Between the spaces there was a fibrous-cellular stroma,



Figures 1 to 4

(See legends on opposite page)

abundant in some spaces and scanty in some others. Among the stroma cells two different cell types could be recognized. One type was a spindle-shaped or polygonal cell, with a large pale-stained nucleus, a fine chromatin network, one or two nucleoli and abundant cytoplasm. The other type was a small round or oval cell with a dark nucleus and a thin ring of cytoplasm. The chromatin was mostly condensed at the periphery of the nucleus, forming a distinct nuclear membrane. The surrounding cytoplasm was compact in some cells and finely vacuolated in some other cells. Short processes departed from the cellular outlines and showed a tendency to fuse together with similar processes of adjacent cells. These cells, which in many respects resembled the cells lining the sinuses, showed at times a spongy appearance due to abundant sudanophilic granules. In the areas in which the lipopessic cells were most numerous, a network of argentaffin fibers which surrounded almost every cell was shown by the silver-stained sections (Foot). Argentaffin fibers were also present elsewhere but were not so homogeneous in distribution, and the network pattern was disrupted at many points by large hemorrhagic infiltrates. Some of the hemorrhages were recent, others older, and a good deal of blood pigment could be recognized either free or in large mesenchymal cells.

Summary.—In a man aged 59 who died of cardiorenal arteriosclerotic disease, postmortem examination revealed in the liver, the left kidney and the small bowel bluish red spongy nodules which on microscopic examination were found to consist of blood-filled channels lined by endothelial cells in one or more rows. These channels were embedded in a fibrous-cellular stroma rich in reticulum cells and fibers, in young fibroblasts and in histiocytic macrophages containing blood pigment. The vascular tree at a certain distance from the intestinal nodule showed clear evidence of faulty development.

CASE 2.—A 65 year old American-born woman was admitted to the Framingham Union Hospital Sept. 18, 1945, complaining of weakness, dysphagia and sore throat which had been present approximately one month and accompanied by loss of weight. One week previous to admission these symptoms became more severe, with elevation of temperature. There was no mention of serious illness in the past or in the family except for a brother who died of leukemia.

Examination at admission revealed an ill woman, extremely pale. Scattered all over the skin of the arms and the trunk there were numerous purpuric areas,

EXPLANATION OF FIGURES 1 TO 4

Fig. 1 (case 1, from the nodule in the liver).—Intercommunicating vascular spaces lined by endothelial cells lying on a thin ribbon of collagenous fibers. Lymphocytes and fibroblasts, either sparse or in groups, are noticeable in the intervening stroma. Ocular 5, objective 10.

Fig. 2 (case 1, from the nodule in the intestine).—Blood channels and sinus-like formations embedded in a fibrous connective tissue stroma widely infiltrated by fibroblasts and lymphocytes with occasional histiocytes. Ocular 10, objective 10.

Fig. 3 (case 1, from the nodule in the kidney).—Sinus-like spaces lined by endothelial cells in one or more rows, embedded in a fibrocellular connective tissue stroma rich in reticulum cells. Large deposits of blood pigment are present (upper right). Ocular 10, objective 10.

Fig. 4 (case 2, from the mediastinal tumor mass).—Sheaths of reticulum cells embedded in a thick network of argentaffin fibers, in the meshes of which sinus-like spaces are being formed. Ocular 10, objective 40.

well circumscribed and not tender. The tonsils and the tonsillar pillars were edematous and covered with grayish membranes. The cervical and submaxillary lymph nodes were palpable, and the spleen was moderately enlarged.

Repeated examinations of the blood from September 19 to 24 revealed a leukemic picture, with the white cell count ranging from 61,000 to 87,600, and marked anemia of a secondary type. The leukemic cell type was an immature cell with a loose chromatin network, one to three nucleoli, and a large round or bean-shaped nucleus surrounded by abundant basophilic cytoplasm in which azurophil granules could be seen at times. The impression was of a monocytic type of leukemia, and this seemed to be supported by the complete absence from the hemogram of transitional forms, either along the lines of the myeloid cell series or along the lines of the lymphoid cell series.

Roentgen examination showed, besides enlargement of the spleen, a sharply outlined mass extending upward from the lung root to the midportion of the left lung. The nature of this mass could not be determined definitely, but in view of the enlargement of the spleen and of the hematologic findings, it was thought to be a lymphoma.

The condition of the patient rapidly became worse, with repeated vomiting of large amounts of dark brown liquid material and with development of new tender ecchymotic areas on both arms and legs. Six days after admission the patient died.

Necropsy.—The body was that of a well developed, somewhat obese white woman with petechial hemorrhages disseminated throughout the body, most numerous at the upper and lower limbs. Moderate pitting edema was present at both ankles and at the dorsal region of the feet. The spleen and the liver could be palpated, and they appeared to be markedly enlarged. There was general lymphadenopathy of slight degree, most apparent at the neck and at the axillary and inguinal regions.

There were no adhesions or fluid in the abdominal cavity. The spleen was enlarged and moderately firm in consistency, and it weighed 350 Gm. The liver also was enlarged and extended 3 inches (7.5 cm.) below the costal margin at the level of the xiphoid process. It weighed 2,000 Gm., and both externally and on the cut section it displayed streaks of pale gray which stood out from the yellowish brown color of the hepatic parenchyma. The kidneys weighed 300 Gm. together and were not unusual externally, but when cut through they showed the limits between cortex and medulla to be indistinct, owing to a rather homogeneous grayish pink color throughout. None of the other abdominal organs showed significant changes.

When the thoracic cavity was opened, a large mass was seen to occupy most of the anterior mediastinum and to cover the upper third of the cardiac region. This mass, round in shape and 5 inches (12.5 cm.) in diameter, was loosely attached to the inner margin of the right lung; it was firm in consistency and slightly lobulated, and both externally and on the cut sections had a markedly variegated appearance owing to alternating grayish pink and spongy reddish brown areas.

The lungs and the heart were essentially normal except for patchy atherosclerotic changes in the coronary vascular tree.

Microscopic Observations.—Representative sections of the principal organs of the body when studied histologically made us modify our impression of the nature of the leukemic process. The liver, the spleen, the myocardium, the kidneys and the lymph nodes were found to be extensively infiltrated by leukemic cells. Quite

a number of these cells were young, immature blood cells, but all progressive stages of differentiation along the lines of myeloid cells could be recognized. Large cells, mononucleated or multinucleated, with characteristics of megakaryocytes were recognizable in the leukemic infiltrates, most numerous in the spleen and the lymph nodes. The myelogenous type of the leukemic process was further shown by the smears from the bone marrow, which displayed a predominance of myeloblasts and myelocytes.

The microscopic sections from the mediastinal tumor mass unexpectedly revealed it to be independent of the leukemic process. A loose interlacing of connective tissue fibers, in the meshes of which lay numerous blood capillaries and young mesenchymal cells, was the main feature of the growth. In some areas the vascular structure prevailed, while in some others the proliferation of mesenchymal cells was predominant (fig. 4). The majority of the blood vessels appeared as sinus-like spaces lined by a regular row of flattened endothelial cells, while elsewhere they showed a more evolved structure, as revealed by a distinct fibrous membrane in which muscular fibers could be recognized. Swelling of the lining endothelial cells and at times actual endothelial cell proliferation could be noticed in places, resulting in cellular processes protruding into the sinus spaces. Sheaths of cells identical in character could be recognized in the surrounding fibrous connective tissue stroma. A linear fissure or a larger opening was at times noticeable in the thickness of these cellular sheaths, and red blood cells well separated one from the other could be recognized in what appeared to be the earliest attempt at canalization of these newly formed endothelial buds. These sheaths of endothelial cells were embedded in a thick network of argentaffin fibers which spread throughout in spider-like fashion.

Dilatation and engorgement of the newly formed blood vessels often resulted in a cavernous-like tissue, and it was mainly in these areas that extravasated red blood cells and extracellular and intracellular blood pigment could be demonstrated.

Among the cells in the cellular portion of the growth, three different types were recognized. One type was an elongated, spindle-shaped cell with a large pale-stained vesicular nucleus, identified as a fibroblast. Another was a large round or polyhedral cell with thick short cytoplasmic processes which showed a tendency to fuse with similar processes of identical adjacent cells or with the pericellular argentaffin reticulum. Its nucleus showed a fine chromatin network, one or two nucleoli and a distinct nuclear membrane which was surrounded by abundant compact and basophilic cytoplasm. Its characteristics were those of a reticulum cell. While these two cell types were irregularly distributed and mixed, the third cell type showed a tendency to nodular arrangement, in groups of 20 to 30 cells or more. Under the low powers these cells resembled lymphocytes, but under the higher powers they were found to include plasma cells and quite numerous hematic blast cells with all transitional stages in the myeloid cell series. The possibility that these cells entered the tumor from the hematic stream could not be ruled out; however, in favor of their local origin were their frequent independence of the walls of the blood vessels and their localization in relatively avascular areas.

Summary.—In a woman aged 65 who died of myelogenous leukemia, postmortem examination revealed a mediastinal mass composed mainly of endothelial-lined sinuses and of impervious or canalized endothelial sprouts. These structures were embedded in a fibrocellular stroma

which displayed widespread proliferation of reticulum cells and fibers, fibroblasts and immature hematic cells. There were concomitant red blood cell extravasations, and blood pigment could be noticed in places.

CASE 3.—A 17 year old white American-born boy was admitted to the Framingham Union Hospital Dec. 25, 1945. He complained mainly of an ache in his left leg which radiated down below the knee. This pain was described as a steady ache which persisted even while he was lying perfectly still in bed. Two weeks before he had had similar pains in his right shoulder, but they were immediately relieved by lamp treatment.

Examination at admission encountered essentially normal conditions, and the diagnosis was "neuritis of the sciatic nerve." On January 12 an enlarged lymph node was noticed in the left supraclavicular fossa. It increased steadily in size during the following days, in spite of intensive penicillin treatment. No nodes were palpable elsewhere in the body, and the spleen and the liver were not enlarged. Repeated hematologic studies failed to reveal any significant deviation from the normal. The white blood cell count ranged between 6,000 and 8,300, with a hemogram within normal limits. The hemoglobin ranged between 17.5 and 14.5 Gm., and the red blood cell count between 5,000,000 and 4,100,000. The Hinton test was negative, as were the heterophilic antibody reaction and the Widal reaction for typhoid and paratyphoid. Blood chlorides amounted to 374 mg. per hundred cubic centimeters; serum amylase, 16 units (normal 16 to 64 units). The urine and the spinal fluid were essentially normal. Repeated determinations of the sedimentation rate showed it to be consistently accelerated. Roentgen studies failed to reveal any significant change except for fusion of the spinal processes of the first sacral segment with narrowing of the disks.

January 15 the supraclavicular lymph node was removed for histologic study. The specimen consisted of a firm bean-shaped formation which measured 3 by 1.5 by 1 inches (7.5 by 4 by 2.5 cm.). It was smooth, grayish pink and glistening externally, while on the cut section it showed a slightly lobulated appearance and a variegated color due to alternating pale gray and yellowish areas. On the microscopic sections the usual lymph-glandular structure was almost completely obliterated and replaced by a widespread proliferation of large polyhedral pale-stained cells with abundant basophilic cytoplasm and vesicular nuclei, rich in chromatin. Cells with two nuclei and occasional mitotic figures were present (fig. 5). These cells were enmeshed in a delicate reticulum of argentaffin fibers, more abundant around the walls of the lymph sinuses. From the fusion of these cells, large cellular sheaths were being formed. Fibroblasts were noticeable here and there, and granulocytic cells, either sparse or in small groups, were also present. The lymph sinuses were dilated and lined by swollen endothelial cells, many of which lay free in the lumens. The capsule of the lymph node was somewhat swollen and edematous but did not show evidence of cellular infiltration. Smears and cultures of the lymph node failed to reveal pathogenic micro-organisms. The pathologic diagnosis was a reticulum cell growth, aggressive in character.

Five days after the removal of the lymph node, pains developed in the upper part of the abdomen, more marked on the right. The entire abdomen was tender; there was a good deal of voluntary spasm, but at no time was involuntary spasm elicited. The abdomen was moderately distended, but no masses could be felt. The patient started to vomit frequently and looked severely ill. An exploratory laparotomy was considered, but in view of the development of a pericardial friction rub the operation was postponed. The condition of the patient became worse, and he died January 26.

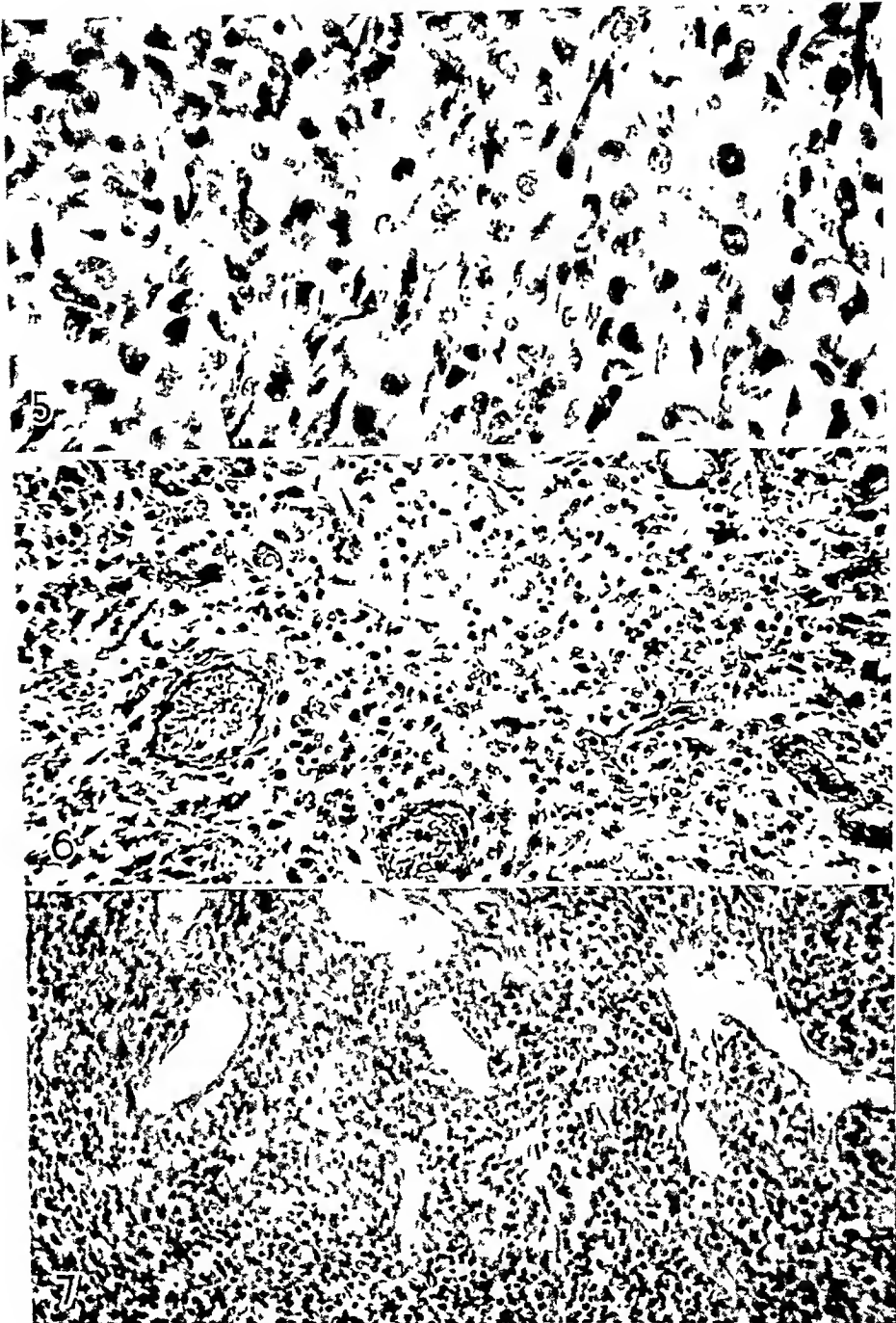


Fig. 5 (case 3, from the supraclavicular lymph node removed at biopsy).—Widespread proliferation of large, polyhedral, pale-stained reticulum cells with abundant basophilic cytoplasm and vesicular nuclei rich in chromatin. Cells with two nuclei and occasional mitotic figures are present. Ocular 10, objective 40.

Fig. 6 (case 3, from the intestinal nodule).—Thin-walled capillaries embedded in a fibrocellular stroma rich in large reticulum cells. Ocular 10, objective 10.

Fig. 7 (case 3, from the nodule in the liver).—Closely packed reticulum cells, irregularly mixed with lymphocytes and fibroblasts, embedded in which sinus-like formations containing red blood cells can be recognized. Ocular 10, objective 10.

Necropsy.—The body was that of a well developed but poorly nourished young man who appeared to be somewhat older than the stated age of 17 years. No injury or lesions of any type were noticeable in any part of the body except a slight pitting edema at both ankles. When the body was opened no significant changes were found in the organs of the chest. The lungs were free from adhesions and well aerated throughout; the heart was not unusual in shape and weighed 320 Gm. The parietal endocardium of the left auricle showed patchy fibrous thickening, and the mitral leaflets were moderately swollen and edematous, with small fibrous plaques at the free borders. The great omentum was retracted and both large and small intestines were distended by gas. There was no fluid in the peritoneal cavity, but both the parietal and the visceral peritoneum were congested throughout and slightly dull in appearance. A segment of ileum, 4 feet (about 1 meter) above the ileocecal valve, was coated by a thin layer of yellowish, easily detachable fibrinous material. On further examination of this segment of intestine, a portion of ileum was found to be invaginated into another portion of ileum. The intussusceptum measured 10 inches (25 cm.) in length. Both externally and on the mucosal side, the invaginated portion displayed extensive gangrenous hemorrhagic changes. A moderately firm nodule, 0.5 inch in diameter, grayish pink in color and spongy in appearance, bulged beneath the mucosa. Its gross appearance was not that of a polyp, and evaluation of its nature was left to the microscopic examination. The intussusciens surrounded the intussusceptum tightly, and was markedly congested and coated with a fibrinous exudate.

A nodule somewhat larger than that found in the intussuscepted intestinal loop, 3.4 inches (8.5 cm.) in diameter, but identical in character, was present in the liver. The latter was a rather large organ, not unusual in shape, which weighed 1,600 Gm. The grayish pink color of the nodule, which was embedded in the posterior border of the right lobe, contrasted sharply with the yellowish brown color of the surrounding hepatic parenchyma. The spleen also was moderately enlarged and weighed 250 Gm. It was firm in consistency and showed pulp increased in amount. Examination of the inguinal and axillary regions revealed an enlarged lymph node in the left axilla. It was the size of a bean, somewhat firm in consistency and homogeneously yellowish gray both externally and on the cut section. A few enlarged lymph nodes, similar in character, were found at dissection of the mesentery.

Microscopic Observations.—The microscopic study was centered in the nodule found in the intussuscepted portion of the intestine and in the hepatic nodule. The outstanding feature in both was an irregular network pattern resulting from the interlacing of connective tissue fibers, which were thick in some areas and thin in others, merging into broad bands of amorphous, amphophilic, nonfibrillar tissue. Elsewhere this network pattern was not so distinctly recognizable, owing to a dense infiltrate of cells which, although they showed great variations in size and shape, displayed a quite uniform intimate structure. The nuclei were large, round or oval, with a pronounced vesicular appearance. The chromatin was mostly condensed at the periphery of the nucleus, forming a distinct nuclear membrane. There were cells with several large nucleoli, and often two nuclei were seen. The surrounding cytoplasm was homogeneous, dark and compact in some cells, palely stained and finely vacuolated in some others owing to the presence of small fat droplets (sudan). Short cytoplasmic processes departed from the cell periphery to fuse either with similar processes of adjacent cells or with the network of collagenous and precollagenous fibers (Foot method) on which they rested. In other areas no intercellular material could be seen, and the cells of the growth appeared closely

packed, with loss of cellular outlines and a tendency to fuse together in large cellular sheaths. Sinus-like formation containing red blood cells or thin-walled capillaries were at times noticeable in the thickness of these compact cellular areas (fig. 6, from the intestinal nodule). Some of these vascular spaces seemed to lie in direct contact with the elements of the cellular bed in which they lay without the interposition of any other cellular type or fibrillar structure (fig. 7, from the nodule in the liver). Some others showed a more or less regular lining of flattened or spindle-shaped cells, whose elongated vesicular nuclei contrasted sharply with the dark-stained round nuclei of the surrounding cells. The cellular elements lining these spaces were disposed at times in a discontinuous single-layered row; at other times they showed overlapping, giving rise to cellular spurs protruding into the sinus spaces. Large hemorrhages and deposits of blood pigment, either free or in macrophages, were present.

In both the hepatic and the intestinal localization the tissue new growth was accompanied by extensive damage of the local structures. There was complete obliteration of hepatic cells in some areas, while in some other areas the hepatic cords appeared spread apart or atrophied with loss of any trace of acinous arrangement. The same was true of the nodule in the intestine, where, in addition, the tumor picture appeared overshadowed at many points by tissue necrobiosis and by infiltration of the tissue by inflammatory cells in concomitance with the intestinal intussusception.

Changes identical with those characterizing the hepatic and the intestinal nodule could be recognized not only in proximity to, but also at a certain distance from, the nodules themselves. As neither the lymphatic nor the hematic blood channels showed evidence of invasion by tumor structures, the impression was that these extranodular lesions were not metastatic but independently originated.

Among the changes in the other organs, those encountered in the spleen and in some axillary and mesenteric lymph nodes are worth mentioning. In the spleen the limits between pulp and follicles were rather poorly preserved. This was mostly due to a diffuse hyperplasia of reticulum cells, equally affecting the lymph follicles and the pulp in broad areas. In the lymph follicles the hyperplasia of reticulum cells involved the central portion most conspicuously. This appeared to be replaced by pale-stained large cells, round or oval, with short and drawn-out acidophil cytoplasmic processes. Their nuclei were oval, with unfolded nuclear membranes and a scanty fine chromatin network containing small nucleoli at times. Two nuclei were often present. Cells identical in type were seen in the pulp. The arrangement of the cells varied greatly. There were areas in which the cells lay in close proximity, with scanty intercellular material. Elsewhere the cells lay in nests, forming an alveolar or mosaic arrangement which gave the appearance of an epithelial growth. In other places they were disposed in rows, with a tendency to form intersecting trabeculae. The histologic resemblance of these cells to the cells lining the sinuses appeared unquestionable, and the finding was interpreted as early attempts at sinus formation. The sinuses were engorged and showed swelling and at times actual proliferation of the endothelial linings, accompanied by abundant cellular desquamation. Extensive extravasations of red blood cells were noticeable in places. Some were recent and others old, with large deposits of blood pigment either free or contained in large histiocytic macrophages.

A fundamentally identical structure was shown by the axillary and mesenteric lymph nodes, many of which displayed hyperplasia of reticulum cells in broad areas with reduction of lymphocytes. Cells with two nuclei and occasional mitotic

figures could be recognized among the proliferated reticulum cells, which displayed a marked tendency to syncytial arrangement. The lymph sinuses were dilated and showed pronounced swelling and desquamation of the endothelial linings.

Summary.—In a boy aged 17 years who died of intestinal intussusception postmortem examination revealed a bluish red spongy submucous nodule in the intussuscepted portion of the intestine. A similar nodule was present in the liver. Both were found to be composed of sinus-like spaces and thin-walled blood capillaries embedded in a cellular stroma mostly composed of reticulum cells and fibers. Large hemorrhages and deposits of blood pigment were present. Changes identical with those characterizing the intestinal and hepatic nodules were noticeable in proximity to, and at a certain distance from, the nodules themselves, and were interpreted as being independently originated. There was concomitant enlargement of the spleen and of the lymph nodes, which showed a striking proliferation of reticulum cells with patterns suggesting early attempts at sinus formation.

COMMENT

The subdivision of vascular growths into nonneoplastic, noncancerous neoplastic, and cancerous is generally accepted (Aegerter and Peale⁹). To the first group belong varicosity, telangiectasia, cirroid aneurysm, phlebarteriectasia, diffuse phlebectasia of the limbs and congenital arteriovenous fistula, all characterized by a malformative process consisting either in faulty distribution or in faulty development of the blood vessels in a circumscribed area of the body.

A quantitative and, to a certain extent, qualitative disorder of vascularity is characteristic of the group of noncancerous neoplastic growths, exemplified by angioma simplex and by angioma hypertrophicus of Zigler, capillary or cavernous, all of which are based on a benign new formation of vascular structures.

Anaplastic and disorderly proliferation of blood vessels, accompanied by a more or less aggressive new formation either of periadventitial connective tissue cells (angiosarcoma) or of endothelial cells lining the intima (angioendothelioma, capillary endothelioma), is the feature of the cancerous growths.

Although it is provided with a lower grade of malignancy, hemoangioblastoma has recently been added to the group of cancerous growths. It is characterized by the presence of pseudo-xanthomatous cells embedded in the meshes of a reticulum of argentaffin fibers in the interstices of the newly formed blood vessels. "Solid hemoangioblastoma" is the term given to the growths in which cellular proliferation prevails; the growths in which vascular proliferation, under various forms, is predominant are called cystic capillary or cavernomatous hemangioblastoma.

This being the accepted scheme of classification, there are a number of cases of vascular growth reported in the literature which, although belonging to the group, hardly fit in any of the categories. This is true for our cases, in which new formation of blood channels in all localizations was the most striking but not the only feature. They showed proliferation of endothelial and adventitial connective tissue cells, phagocytic histiocytes, fibroblasts, lymphoid elements, a well organized reticulum and in 1 case immature hematic cells, as well.

In the past, one gathers, such growths have been classified among the hemangioendotheliomas, on the assumption that only the prolific vascular endothelium could give rise to such structures. Considering that the vascular endothelial cell is nothing more than a functional adaptation of the pluripotent periadventitial cell, we feel that this cell and not the product of its differentiation must be considered as the basic cellular unit involved in the process. For the same reason we do not agree with the conception of Orsós³⁸ who, in describing a number of vascular tumors, some composed partly of blood capillaries and partly of endothelial sprouts, and some much more complex, with both capillaries and immature hematic cells, concluded that blood vessels and blood cells had originated from an embryonal angioblastic cell. The term "angioblast" proposed by Orsós implicitly limits the developmental possibilities of this embryonal cell, which in our opinion has much broader evolutive possibilities. The old conception of Albrecht of a developmental defect underlying a number of vascular growths has been stressed more recently by several authors. An inclusion of angioblastic structures occurring in the central nervous system is advocated by Roussy and Oberling³⁹ to explain the development of certain vascular tumors of the brain. The disembryoblastic origin of Lindau's disease is apparent, and the fact that it is not infrequently accompanied by other organic malformative processes had made some consider the possibility of a more general developmental defect (Tedeschi⁴⁰).

In a case of cavernous degeneration of the liver that one of us had the opportunity of studying (Tedeschi), the vascular defect extended beyond the cavernous area and the whole vascular structure of the liver appeared to be disarranged; in another case of angiomatosis of the liver observed by the same author, the disembryoblastic nature of the process was emphasized by a peculiar syncytial arrangement of the hepatic cells, by the apparent absence of any endothelial lining in many of the sinusoidal spaces and by the paucity and irregularity of distribution of the reticulum of argentaffin fibers. A case somewhat similar

38. Orsós, F.: Beitr. z. path. Anat. u. z. allg. Path. **93**:121, 1934.

39. Cited by Tedeschi.⁴⁰

40. Tedeschi, C. G.: Riv. sper. di freniat. **56**:497, 1932.

to this was described by Lunghetti,³⁹ that of a telangiectatic liver in which structural changes were clearly noticeable in both arteries and veins beyond the telangiectatic areas.

In describing a familial telangiectatic dysplasia of the Osler type, Weber⁴¹ alluded to the possible analogy and association of the telangiectatic condition of the skin and mucous membranes of the nose and the mouth with certain hemorrhagic telangiectatic conditions of the stomach, the intestines, the kidneys or the lungs. The telangiectasia in this disease he regarded as due to a congenital developmental dysplasia of the small vessels, potentially present at birth though often not manifesting itself till after puberty.

The possibility that a malignant vascular growth originates from a localized debility in the vascular system is shown by a number of cases in the literature. In the hemolymphangiosarcoma of the liver described by Pepere³⁹ the hepatic capillary network obviously showed deviations from the normal in areas situated far beyond the new growth. In a similar observation by Mark³⁹ a hemoangiosarcoma of the liver appeared to have originated from a preexisting cavernous angioma. The same was thought to be true of a cavernoma of the spleen described by Wright,³⁹ which showed proliferation both of lining endothelial cells and of interstitial mesenchymal cells; an identical structure was shown by a hepatic nodule that was considered to be metastatic. In discussing an unusual tumor of the liver, characterized by multiple nodules composed of blood capillaries, endothelial cells in atypical proliferation, giant cells and foci of extramedullary hemopoiesis, Fisher³⁹ concluded that the growth had originated in a faulty development of the capillary bed of the organ.

A similar interpretation might be offered for our cases, for which it is logical to suppose that the growths started independently on the ground of a focal vascular debility, congenital or acquired. However, considering the number of structures involved in the growth, we should locate this debility not in the vascular system but in the mesenchymal syncytium, the only system in the body which under the influence of adequate stimuli is potentially able to differentiate itself into the many lines of development shown by the growths in the different localizations.

Histologic evidence to support this belief was found in what appeared to be transitional forms from reticulum cells and the flat endothelial cell forerunners of the vascular channels, in the demonstration of fine processes interpreted as early attempts at formation of the fibrillar reticulum noticed on the surface of the cytoplasmic reticulum, and in the formation of hematic cells (case 3) which seemed to be initiated by a basophilia developing in the perinuclear zone of the cytoplasmic syncytium. Simultaneously, the nucleus showed coarser chromatin

41. Weber, F. P.: *Brit. J. Dermat.* 48:182, 1936.

granules, and the nucleoli became more conspicuous, the result being the formation of a cell with the cytoplasmic and nuclear characteristics of a hemocytoblast. Some of these cells still possessed stellate processes, but others became gradually rounded off and through progressive stages they assumed the characteristics of myeloblasts and myelocytes.

Foci of hemopoiesis in vascular tumors have been reported in a number of cases. In that of Fisher,³⁹ immature hematic cells could be recognized in hepatic nodules composed of sheaths of endothelial cells and of blood channels resting in a fibrous connective tissue stroma, embedded in which were young mesenchymal cells, scattered throughout. A tumor in the liver described by Pilliet and Schmieden³⁹ and one of the dura mater described by Albrecht³⁹ showed similar structures. Under the title *kombinierte Formen* Roulet⁴² described reticulum cell tumors of different organs, in which either leukemic or lymphogranuloma-like features were concomitant findings. Similar observations in lymph-glandular structures were reported by Vecchi⁴³ and by Montpellier, Manceaux and Assan,⁴⁴ all authors agreeing in the belief that there was an atypical growth evolving in more than one direction from an undifferentiated stem cell. Accordingly, Oberling⁴⁵ and later Craciun and Ursu⁴⁶ have recognized in the broad group of reticulum cell tumors myelogenous reticulomas and lymphogenous reticulomas, characterized by striking differentiation of the pluripotent parent reticulum cell into myelogenous or lymphatic cells.

The question arises whether the nodular lesions found in various organs in our 3 cases can be identified from the histologic standpoint with the cutaneous nodular manifestations of Kaposi's disease. After close comparison between the findings in our cases, the findings reported by other students and personal observations on biopsy material from cutaneous nodules, our impression is that the patterns of the visceral nodules in our cases exactly overlap the patterns of the cutaneous nodules in Kaposi's disease. Choisser and Ramsey,² in discussing their cardiac new growths, arrived at a similar conclusion; however, in view of the absence of cutaneous lesions they felt that the morphologic diagnosis of angioreticoendothelioma was better than that of Kaposi's disease. We felt the same at the time when cases 1 and 2 were being investigated, but on further study of these 2 cases and the third similar case, having reached the conviction that the visceral lesions in our cases were identical with those of cutaneous Kaposi's disease, we no

42. Roulet, F.: Virchows Arch. f. path. Anat. **277**:15, 1930; **286**:702, 1932.

43. Vecchi, G.: Cancro **5**:45, 1934.

44. Montpellier, J.; Manceaux, A., and Assan: Gaz. méd. de France, 1931, p. 667.

45. Oberling, C.: Bull. Assoc. franç. p. l'étude du cancer **17**:259, 1928.

46. Craciun, E. C., and Ursu, A.: Bull. Assoc. franç. p. l'étude du cancer **22**:711, 1933.

longer see why, in a systemic process likely to involve any part of the body and to affect independently, simultaneously or successively both skin and internal organs, all manifestations, whatsoever their localization, should not be covered by the same label. Eponyms, it is true, are not often desirable, yet they might be in regard to a disease process such as the one under consideration, characterized by a great variety of histologic patterns which are inherent to the multiple developmental potentialities of the basic unit of growth, the reticulum cell.

The dispute over the hyperplastic or dysplastic nature of Kaposi's disease, which has been carried on since the early description of the process, is, in our opinion, futile. Both hyperplastic and dysplastic growths can be expected. A hyperplastic type of growth, corresponding to the inflammatory or granulomatous stage of MacKee and Cipollaro's classification,⁴⁸ will occur whenever the differentiation of the unit of growth into various cell types proceeds harmoniously along the lines of normal development, with fairly well balanced proliferation of vascular elements and supporting fibrocellular stroma; on loss of this organizing power a dysplastic type of growth will occur, provided with wild and aggressive potentialities, and if the vegetative function of the cell type involved in the process prevails, it will result in a solid cellular tumor, hardly distinguishable from a sarcomatous growth.

There are a number of visceral growths reported in the literature which simulate closely those observed in our cases. The splenic hemangiosarcoma most recently described by Bauer and Stanford,⁴⁷ mainly composed of blood-filled channels lined by a stratified layer of cells, of a thick network of reticulum fibers and of a cell type which in some places simulated fibroblasts, elsewhere macrophages, and in still other places endothelial cells lining the normal splenic sinuses, exemplifies many similar pleomorphic vascular growths recorded in the literature under a number of names. In keeping with the fundamental structure of Kaposi's disease these growths might be explained on the basis of a differentiation along various lines of the pluripotent reticulum cell. The tonsils, the bones, the vertebral bodies and the liver were involved by an identical process in the case of Bauer and Stanford.⁴⁷ This was interpreted as metastatic, and it may have been metastatic, but it cannot be disproved that there may have been an autonomous origin underlying a systemic involvement of the reticulo-endothelial system. This explanation might apply also to the so-called metastasizing hemangioma (Robinson and Castleman⁴⁸; Ward and Jones⁴⁹) and to the angioblastic or hypertrophic type of hemangioma,

47. Bauer, D. F., and Stanford, W. B.: *Arch. Path.* **41**:668, 1946.

48. Robinson, J. M., and Castleman, B.: *Ann. Surg.* **104**:453, 1936.

49. Ward, G. E., and Jones, A. F.: *Ann. Surg.* **36**:330, 1938.

often mistaken microscopically for hemangiosarcoma and as a matter of fact frequently observed to be locally aggressive and prone to recur after operation (Watson and McCarthy⁵⁰). A good number of these growths might fit, in our opinion, in a broader conception of Kaposi's disease, namely that of a systemic process of the reticuloendothelial system likely to involve independently and in an unpredictable manner, simultaneously or successively, the skin and the internal organs and characterized from the histologic standpoint by a great variety of patterns which are inherent to the multiple developmental potentialities of the basic unit of growth, the reticulum cell.

SUMMARY

The literature on Kaposi's disease is reviewed and analyzed with special reference to the visceral manifestations. From the study of 2 cases in which the liver, the kidney and the small bowel showed multiple independent growths and of a third case with mediastinal localization, all the growths consisting of newly formed blood channels and including proliferations of endothelial and adventitial connective tissue cells, of phagocytic histiocytes, of fibroblasts, of lymphoid elements, of a well organized reticulum and, in one case, of immature hematic cells, the conclusion is reached that the patterns of the visceral lesions in these cases were identical with those of the cutaneous lesions of Kaposi's disease. The conception is advanced that Kaposi's disease is systemic in nature and likely to involve independently and in an unpredictable manner, simultaneously or successively, the skin and the internal organs and that it is characterized from the histologic standpoint by a great variety of patterns inherent to the multiple developmental potentialities of the basic unit of growth, the reticulum cell.

50. Watson, W. L., and McCarthy, W. C.: Surg., Gynec. & Obst. **71**:569, 1940.

FIBROSARCOMA OF THE EPIDIDYMIS

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NEOPLASMS originating in the epididymis are rare.¹ According to Charache,² 22 benign and 54 cancerous neoplasms of the epididymis were recorded up to 1939. Charache combined the cases collected by Scalfi³ with those tabulated by Lazarus.⁴ Among all these 1 was listed as an instance of spindle cell sarcoma and 1 as an instance of fibrosarcoma. The fibrosarcoma, described by Lazarus, occurred in a patient 44 years old. The only one reported since is that described by O'Brien,⁵ in a patient 20 years old. The case herein presented is believed to be the third recorded instance of a fibrosarcoma of the epididymis and the first of fibrosarcoma occurring in early childhood.

REPORT OF A CASE

A 3 year old white boy was admitted to the University of Oklahoma Hospitals, Sept. 18, 1945, with a mass in the right side of the scrotum, first noticed in June 1945. The mass enlarged rapidly and became painful a few days before admission. There were no other genitourinary symptoms. The child was well developed and well nourished. Except for the enlargement of the scrotum, no abnormalities were noted. The left testicle was of usual size, and the testis and epididymis could be felt. The content of the right side of the scrotum was replaced by a firm, smooth, slightly tender mass, 7 by 5 cm., with the epididymis not palpable. There was no increase of local heat. The mass could not be transilluminated.

Urinalysis gave essentially negative results. The red blood cell count was 4,750,000; the hemoglobin content was 13 Gm.; the white blood cell count was 5,500, with polymorphonuclears 55, lymphocytes 35, eosinophils 5 and monocytes 5 per cent. The Mazzini test of the blood was negative. On September 22 the content of the right side of the scrotum including the spermatic cord up to the level of the external inguinal ring was removed (by Dr. Herman F. Flanigin).

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1. Halpert, B.: *J. Urol.* **45**:536, 1941. Golden, A., and Ash, J. E.: *Am. J. Path.* **21**:63, 1945. Sworn, B. R.; Marshall, F. W., and Edwards, J. L.: *Brit. J. Surg.* **33**:375, 1946.

2. Charache, H.: *Urol. & Cutan. Rev.* **43**:663, 1939.

3. Scalfi, A.: *Ann. ital. di chir.* **15**:81, 1936.

4. Lazarus, J. A.: *J. Urol.* **39**:750, 1938.

5. O'Brien, M. G.: *J. Urol.* **47**:311, 1942.

The specimen consisted of an oval mass, 8 by 5 by 4 cm., weighing 100 Gm. The external surface was smooth and gray-white. On the cut surfaces there was a gray-white fibrillar, finely striated pattern with no areas of discoloration or hemorrhage. A horseshoe-shaped slitlike space, corresponding to the cavity of the tunica vaginalis propria, surrounded a smooth oval nodule, 2 cm. long, which was continuous at its base, 1 cm. wide, with the rest of the growth (fig. 1).

Microscopic sections taken from various parts of the mass and from the nodule and stained with hematoxylin and eosin disclosed that the mass was the epididymis and the nodule the testis. In the latter the primordial seminiferous tubules were of proportionate size and of usual distribution within the usual amount of fibrous connective tissue. The testis was bordered by the intact tunica albuginea. In the mass, spread apart by neoplastic fibrous connective tissue, were the tubules



Fig. 1.—Cut surfaces of the scrotal mass. The horseshoe-shaped slitlike space corresponds to the cavity of the tunica vaginalis propria. The nodule is the testis, and the mass is the epididymis.

of the epididymis, lined by tall columnar epithelium (fig. 2A). The neoplastic tissue had a delicate fibrillar pattern with various sized, round or elongated, deeply stained nuclei in a streamlike arrangement. The elongated nuclei were blunt or pointed at their ends. Some cells were seen in a state of division. The cytoplasmic borders were not discernible, and the cytoplasm faded into the fibrillar ground substance. The stream or whorl-like arrangement of the cell nuclei and fibrils was more marked in some fields than in others (fig. 2B). Delicate-walled blood vessels were scattered throughout. Minute areas of hemorrhage and necrosis were seen in places.

Roentgen radiation was given over a period of thirty days (October 3 to November 3) to three fields, one including the right inguinal area and the right

half of the scrotum, one located posteriorly over the lumbar region, and one over the sacral area (total dose, 1,800 roentgens to each field; distance, 50 cm.; 200 kilovolts; 18 milliamperes; Thoraeus filter).

On account of local reaction, radiation therapy was discontinued and the patient discharged on November 9. He was followed in the outpatient department until

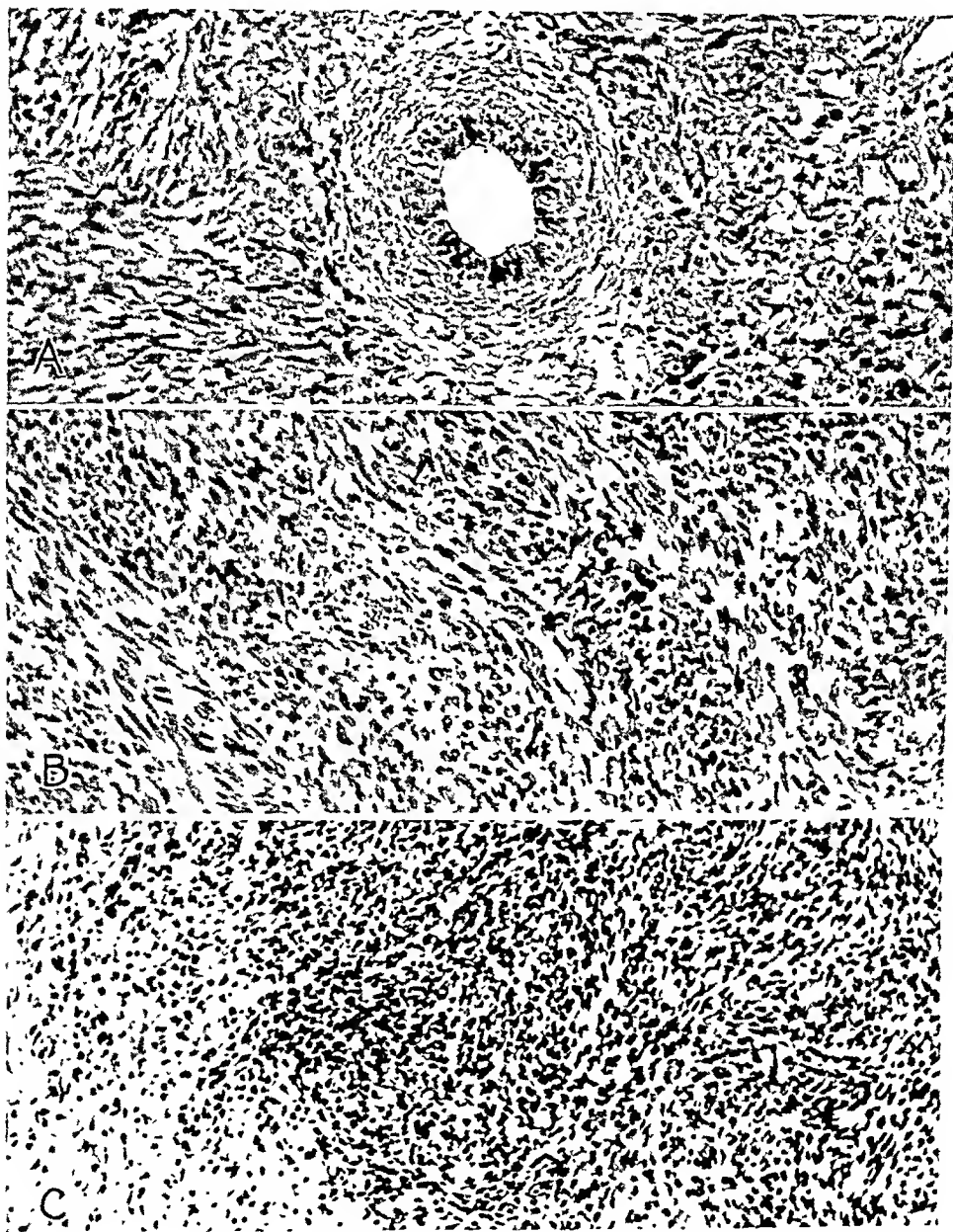


Fig. 2.—Fibrosarcoma of the epididymis. *A* shows a tubule of the epididymis lined by tall columnar cells and surrounded by neoplastic fibrous connective tissue. In *B* the neoplastic tissue is seen to have a delicate fibrillar pattern, with round or elongated, deeply stained nuclei in a streamlike arrangement. *C* reveals that the retroperitoneal mass has a microscopic appearance similar to that of the original growth. $\times 150$.

July 8, 1946, when he was readmitted to the hospital. At this time the complaints were loss of appetite, weakness, constipation, fever and pain in the right side of the abdomen. The child was emaciated and appeared chronically ill. The temperature was 100 F., the pulse rate 88 and the respiratory rate 20. A healed scar marked the site of operation, and the right scrotum was empty. The left testicle was of usual size. In the right side of the abdomen a firm, slightly tender mass was felt, extending from the iliac crest to the costal margin and from the flank to the midclavicular line.

The urine was yellow, cloudy, with a p_H of 7.5; it revealed no albumin and no sugar. The red blood cell count was 3,470,000; the hemoglobin content was 8 Gm.; the white blood cell count was 10,650, with polymorphonuclears 73 (stab forms 4), lymphocytes 24, eosinophils 2 and monocytes 1 per cent. Intravenous urography on July 23 failed to visualize the pelvis of the right kidney; that of the left seemed as usual. Cystoscopic examination, July 27, disclosed no lesions in the urinary bladder. An attempt to pass a catheter through the right ureter was unsuccessful. The abdominal mass continued to enlarge, and the abdomen became distended with fluid. The temperature varied between 96 and 103 F.; the pulse rate, between 126 and 144. There was rapid loss of weight and strength. Supportive measures were of no avail, and the child died August 10.

Necropsy (eight hours after death).—The body was 100 cm. long and weighed approximately 30 pounds (13.5 Kg.). The face had a wizened appearance. The neck and the chest were symmetric. The skin was tightly drawn over the ribs. The abdomen was prominent and tense. There was a scar 3 cm. long to the right of the root of the penis. The content of the right scrotum was missing; the left testis was of usual size. There was marked wasting of the flesh of the upper and lower extremities, with the skeletal markings prominent.

The peritoneal cavity contained 750 cc. of light milky fluid. The greater omentum was gray-white and up to 2 cm. thick, and its free portion was adherent to the anterior abdominal wall and the fundus of the urinary bladder. The edge of the liver was 5 cm. above the costal margin in the right midclavicular line. The surface of the diaphragm was studded with neoplastic nodules up to 0.7 cm. in diameter which were elevated as much as 0.4 cm. The surfaces of the small intestine and the mesentery were glazed by a thin coating of apparently neoplastic tissue. A retroperitoneal mass, 17 by 13 cm., protruded from the posterior abdominal wall, pushing forward the overlying terminal portion of the ileum, the cecum with the appendix, and the ascending colon. It extended behind, and was adherent to, the right kidney without invading it. The mass was extremely friable and was composed of gray-white tissue mottled with yellow and red. It contained various-sized locules with ragged inner surfaces containing tissue debris, clotted blood and bloody fluid. The mass measured 14 by 10 by 8 cm. after removal and after most of its fluid content had been evacuated.

There was no neoplastic involvement of the pleural and pericardial surfaces. The left testis and epididymis measured 1.5 by 1 cm. and were of usual appearance externally and on their cut surfaces. There was no involvement of the prostate, the urinary bladder, the ureters, the renal pelves, the kidneys or the adrenal glands. The pancreas itself was uninvolved; likewise, the extrahepatic biliary ducts, the gallbladder, the liver and the spleen. There was no involvement of the lungs and of the tracheobronchial lymph nodes.

Microscopic preparations from the greater omentum and from various parts of the retroperitoneal mass disclosed a delicate fibrillar pattern and round or elongated, deeply stained nuclei in a streamlike arrangement similar to that seen in the

original preparation from the epididymis (fig. 2C). There were massive areas of necrosis with old and more recent hemorrhage. No epithelial elements were seen in any of the preparations. No neoplastic involvement was seen in preparations from the lungs, the spleen, the liver, the pancreas, the adrenal glands and the kidneys. The left testis and epididymis had the usual structure corresponding to the age of the patient, and there was no evidence of neoplastic involvement.

COMMENT

The microscopic structure of the original growth and its relation to the testicle leave no doubt that the neoplasm originated in the stroma of the epididymis. The epithelium-lined tubules seen in the original growth were those of the epididymis and were not neoplastic. In the extensions of the growth outside the epididymis there were no epithelial elements. The neoplasm resembled closely Wilms's tumor minus its epithelial elements and was probably derived from a similar anlage.

There was little change in the structural pattern of the growth except that areas of necrosis and hemorrhage became more extensive as the neoplasm increased in bulk. The involvement was by direct extension with no apparent spread by the lymphatics or by the blood stream. It is likely that at the time of removal of the right scrotal content, extension along the spermatic cord into the retroperitoneal tissues had already occurred. The short time, less than one year, which elapsed before the death of the patient indicates the rate of growth of the neoplasm. Had the neoplasm been removed earlier, extension might have been prevented.

SUMMARY

A fibrosarcoma of the right epididymis occurring in a 3 year old boy is reported. The child died of the neoplasm less than one year after surgical removal of the scrotal content. This is believed to be the third recorded instance of fibrosarcoma of the epididymis and the first of fibrosarcoma occurring in early childhood.

SOME MANIFESTATIONS OF VITAMIN E DEFICIENCY IN THE MONKEY

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ONE OR more of the recognized manifestations of vitamin E deficiency (fetal death and resorption, testicular degeneration, muscular dystrophy, accumulations of an inert pigment in the musculature and the reticuloendothelial system) have been observed in a considerable number of laboratory mammals. There appears, however, to be no recorded demonstration of this deficiency state as produced in the monkey. The lack of direct evidence concerning vitamin E deficiency of man emphasizes the need for some knowledge of the reactions of other primates to depletion of vitamin E. The present report records certain observations made on young monkeys maintained for several years on a diet deficient in vitamin E. Although the sexual immaturity of the animals used did not permit any investigation of reproductive functions, other gross and histologic observations, especially those relating to an acid-fast pigment that occurred in certain locations, resulted in findings quite similar to those previously reported for the rat¹ and observed also in other laboratory animals.² These observations are presented as evidence of a state of vitamin E deficiency induced in the *Macaca rhesus* monkey.

MATERIAL AND METHODS

In the course of planning a vitamin E-deficient diet sufficiently palatable for prolonged feeding of monkeys, it was suggested that rice might be utilized as a major constituent. In order to be assured of rice with a low content of the vitamin, several hundred grains from different lots (obtained on the local market

An abstract of these studies was presented before the American Association of Anatomists (*Anat. Rec.* **94**:522, 1946).

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1. (a) Martin, A. J. P., and Moore, T.: *J. Hyg.* **39**:643, 1939. (b) Mason, K. E., and Emmel, A. F.: *Anat. Rec.* **92**:33, 1945.

2. Unpublished studies from this laboratory.

and directly from the millers) were examined with a dissecting microscope and the samples rated in terms of the percentage of grains still possessing the germ or fragments thereof.³ Wide variations were found in the samples examined. When we assayed the lots by rearing newly weaned female rats on vitamin E-deficient diets in which rice from the different lots was fed at a 50 per cent level, those lots rating 10 to 20 per cent consistently produced no fertility responses; samples rating 60 per cent or higher permitted positive responses in some of the test animals. Consequently, rice lots rating 10 per cent or less were selected for purchase and used in the experimental diet. The latter was composed as follows: 500 Gm. of cooked rice, 300 Gm. of skimmed milk powder, 180 Gm. of dry brewers' yeast and 300 Gm. of a vitamin E-deficient diet used for rats (containing casein, 20 per cent; corn starch, 50 per cent; lard, 18 per cent; salts, 2.5 per cent; brewers' yeast, 7.5 per cent; cod liver oil, 2 per cent). The diet was made up twice weekly and stored at 6 to 10 C. until just before feeding, when 25 cc. of orange juice or 25 mg. of ascorbic acid was added to the portion fed each monkey.

Six sexually immature monkeys (3 males and 3 females), estimated to be 2 to 3 years of age and varying in weight from 2 to 2.5 Kg. in body weight, were placed on the experimental diet. One male and one female died after about five months; because of advanced postmortem changes, no tissues were preserved for histologic study. The remaining 4 animals grew well and maintained good health during the first two years of the experiment. Eventually, progressive weakness and gradual loss of appetite, extending over a period of three or four weeks, appeared in 2 animals after approximately twenty-five months and in the other 2 after about thirty-one months. All showed progressive inability to climb or cling to the walls of the cage enclosures. As weakness of the digital flexor muscles became apparent the animals could cling to the walls only by inserting the forearm through the coarse wire mesh; later they refused to climb, remaining relatively inactive on the floor of the cage. During these terminal stages they consumed but little of the experimental diet and would accept only small amounts of bread, milk or orange juice.

One male was found dead after seven hundred and seventy-two days, and 2 females after seven hundred and eighty and nine hundred and fifty-six days, of experimental feeding. An autopsy was made on each within four hours after death. The remaining male was killed on the nine hundred and sixty-sixth day of feeding. In all monkeys, the lymph nodes and the smooth musculature of the gastrointestinal tract showed variable degrees of brownish discoloration, the skeletal musculature appeared somewhat pale and lusterless, and there was considerable depletion of the various fat depots. Only the male that died after seven hundred and seventy-two days of feeding showed evidence of tuberculosis; the lesions were sufficiently extensive to have contributed to the early death of this animal. Except for the dystrophic state of the skeletal musculature, the gross and microscopic changes in the other experimental animals provided no adequate explanation for their decline or death.

Because of the limited number of young monkeys available at the time these studies were begun, and the somewhat exploratory nature of the investigation, no animals were fed the experimental diet supplemented with tocopherol. Instead, 2 male and 2 female monkeys maintained for a year or more on the mixed diet

3. Dr. C. P. Leblond, of the Department of Anatomy, McGill University Medical School, made these suggestions and gave other assistance in the preliminary phases of this study.

of the stock colony' and approximately 5 years of age at autopsy provided control tissues for comparison. The methods of fixation, embedding and staining were the same as for the tissues of the experimental monkeys. With both groups histologic studies were carried out on all visceral organs and on lymph nodes and skeletal muscles from various regions.

Tissues were fixed in Zenker's solution with acetic acid (5 per cent), embedded in paraffin and sectioned at 5 to 7 microns. Sections were routinely stained with (1) hematoxylin and eosin and (2) Verhoeff's or Kinyoun's carbolfuchsin,¹ then destained with acidified alcohol for fifteen to thirty minutes and subsequently stained with Ehrlich's hematoxylin. Staining with Kinyoun's carbolfuchsin (phenol crystals, 8 Gm.; basic fuchsin, 4 Gm.; 95 per cent alcohol, 20 cc.; water, 100 cc.) for one hour at room temperature gave the most satisfactory results. Various other staining procedures were used to permit further characterization of the acid-fast pigment.

EXPERIMENTAL DATA

The pigment referred to throughout this report appeared pale brown in hematoxylin-eosin preparations and varied from brick red to brilliant red after acid-fast staining. It showed moderate affinity for iron-hematoxylin, exhibited a yellowish or yellowish brown fluorescence when examined under the fluorescent microscope, gave a positive Feulgen reaction, and stained yellowish to orange with sudan IV and black with sudan black in paraffin sections (previously exposed to fat solvents). In these various respects it closely resembled the pigment previously described in vitamin E-deficient rats,¹ and since found to characterize vitamin E deficiency in the mouse, the hamster, the cotton rat and the dog.² The general pattern of distribution of this pigment, however, differs considerably in the various species mentioned.

Tissues of the control monkeys were devoid of pigment except for traces of acid-fast material in an occasional macrophage and in cells of the adrenal cortex. The skeletal muscles showed no dystrophic change, but in 2 animals there was considerable *Sarcocystis* infection, as briefly reported elsewhere.⁴ There were no other pathologic changes of note.

One of the 4 monkeys fed the vitamin E-deficient diet showed moderately extensive tuberculous lesions of the respiratory and digestive tracts; otherwise, no significant pathologic developments were observed other than the dystrophic changes in skeletal muscles and the occurrence of acid-fast pigment at various sites as discussed in subsequent sections. While the pattern of the deposition of pigment was the same in all experimental monkeys, the amounts of pigment encountered in the various locations were somewhat greater in the 2 animals continued on experiment for nine hundred and fifty-six and nine hundred and sixty-six days than in the 2 that succumbed after seven hundred and seventy-two and seven hundred and eighty days, respectively.

4. Offutt, E. P., and Telford, I. R.: *J. Parasitol.* (supp.) **31**:15, 1945.

Skeletal Muscle.—The muscles studied were the diaphragm, the rectus abdominis, the pectoralis major, the psoas major, the adductor magnus and the gluteus maximus. All showed degenerative lesions, varying from slight fibrosis with some leukocytic infiltration to rather extensive atrophy and necrosis involving a considerable number of muscle fibers (fig. 1). Only occasionally were pigment granules demonstrable within the degenerating fibers and in macrophages of the supporting connective tissue. However, pigmentation of the vascular smooth muscle, as described in a later section, was extensive in blood

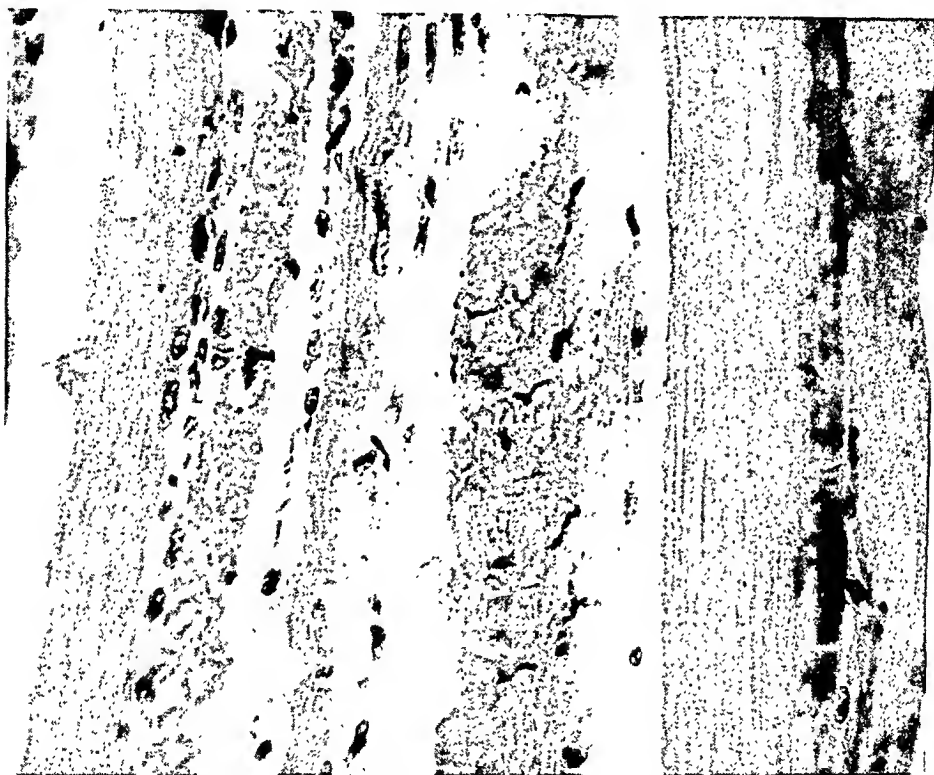


Fig. 1.—Small portion of skeletal muscle (gluteus maximus) showing necrosis of several muscle fibers, accompanied by considerable nuclear proliferation and nuclear pyknosis. Small leukocytes and macrophages are present in the vicinity of the necrotic fibers. Hematoxylin-eosin stain; $\times 430$.

vessels throughout the skeletal muscles. In comparison with the rat, the monkey showed more widespread atrophy of skeletal muscle fibers but proportionately less pigment in these fibers and in adjacent macrophages.

Cardiac Muscle.—Only small and inconstant amounts of pigment were observed in the heart. In 2 monkeys small numbers of pigment granules were observed in the rather clear cytoplasm about the nuclei of the cardiac fibers. Occasional macrophages with pigment accumulations were found along the adventitia of the blood vessels and beneath the

endocardium. Except for some small areas of slight fibrosis, and an occasional necrotic fiber, the cardiac musculature was normal.

Smooth Muscle.—The most striking feature of the various organs and tissues examined was the pigment which had accumulated extensively within the smooth muscle cells forming the media of arteries and veins of all calibers (fig. 2), including arterioles; usually the aorta, the pulmonary vessels and the coronary vessels showed less pigment than other vessels. The granular pigment was sometimes limited to the paranuclear zone but was usually disseminated throughout the cytoplasm of the smooth muscle cells, the latter appearing somewhat swollen

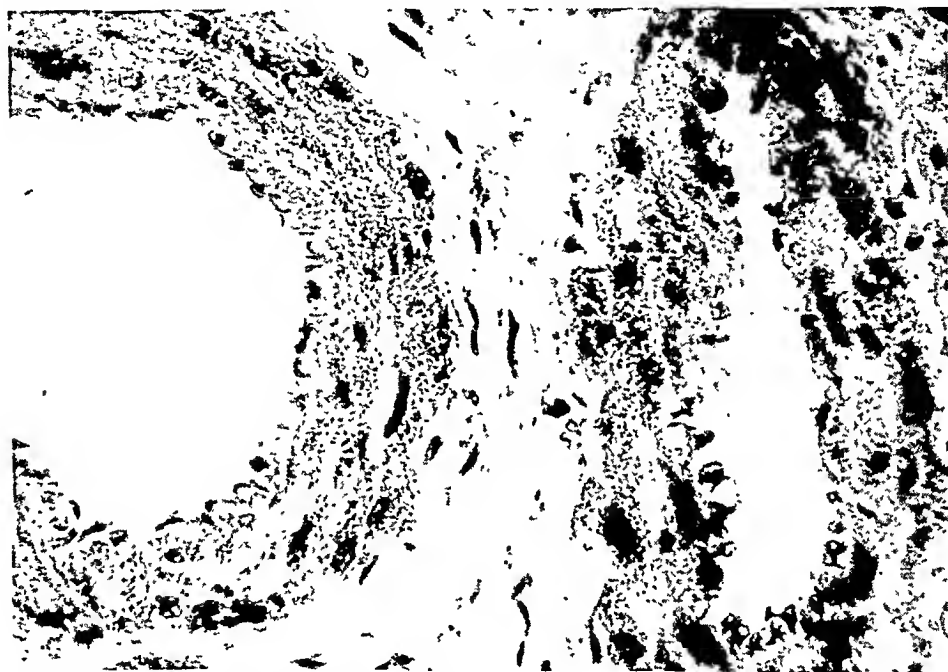


Fig. 2.—Small artery (left) and vein (right) showing abundance of pigment (appearing as black granules) within smooth muscle cells of the media. Acid-fast staining; $\times 430$.

and edematous. As far as could be determined, the vascular endothelium was devoid of pigment.

There was considerable pigment within smooth muscle cells of the muscularis and the muscularis mucosae of the stomach, small intestine (fig. 3) and colon. These changes were more marked in the proximal than in the more distal portions of the digestive tract. The smooth musculature of the gallbladder, bronchi, bronchioles, urinary bladder and ducts of the epididymis was likewise involved to a variable degree; that of the splenic capsule and trabeculae was devoid of pigment.

Pigment was present in moderate amounts in many smooth muscle cells of the muscularis of the fallopian tube but was never identified

within those of the uterine myometrium. In both organs, pigment was abundant in the walls of blood vessels and in macrophages distributed throughout the muscularis and the mucosa. It is of interest in this connection that the uterine smooth muscle and the splenic trabeculae and capsule of vitamin E-deficient rats show rather pronounced pigmentation.^{1b} Small amounts of pigment were observed in the epithelial lining of the uterus and tubes; on the basis of similar findings in other laboratory animals, this material is considered to be of normal occurrence and unrelated to the deficiency state.

Reticuloendothelial System.—The inguinal, internal iliac, mesenteric and tracheobronchial nodes were studied. All showed marked accumu-

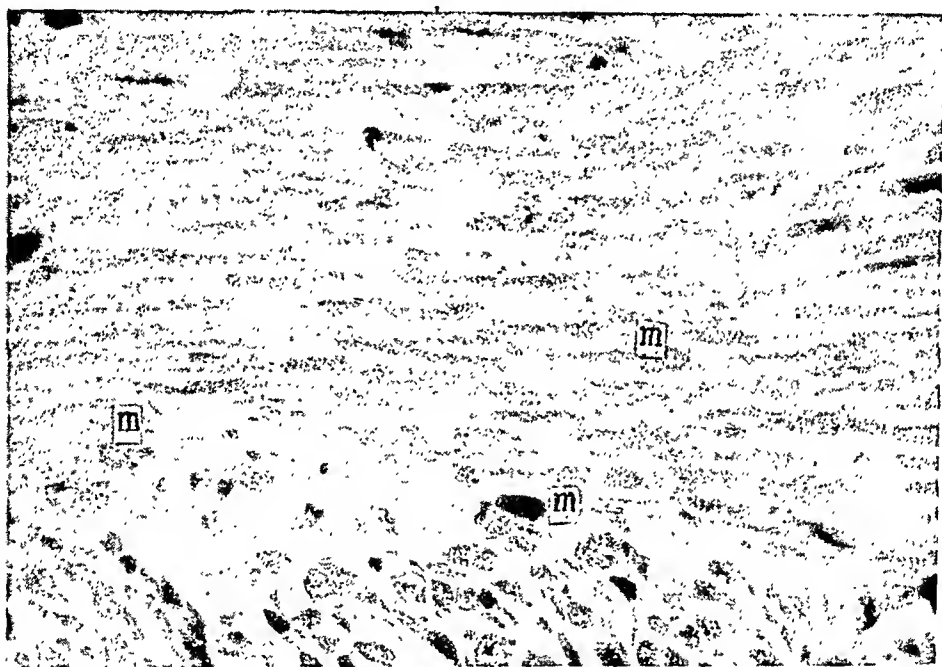


Fig. 3.—Small portion of the circular (above) and longitudinal (below) muscle coats of the duodenum, showing many fine pigment particles within smooth muscle cells and in occasional macrophages (*m*). Acid-fast staining; $\times 430$.

lations of pigment granules and globules in the free macrophages and lining cells of the cortical and medullary sinuses (fig. 4). Sometimes the sinuses were completely filled by these cells. Pigment was also encountered in macrophages scattered throughout the lymph follicles and medullary cords. In some nodes the phagocytic cells contained considerable hemosiderin as well as pigment. Otherwise, the histologic picture was much the same in the various nodes examined. In general, the walls of blood vessels within the nodes and of those in the adjacent connective tissue (fig. 4) contained unusually large amounts of pigment.

In the spleen the accumulation of pigment was less extensive and somewhat more variable than in the lymph nodes. It was localized

principally in the lining cells and macrophages of the venous sinuses but was also abundant in macrophages of the red pulp (fig. 5). Here, too, hemosiderin and pigment occurred sometimes in the same cells and at other times in separate cells. No pigment could be identified in the smooth muscle cells of the capsule or trabeculae, or in the nonmuscular walls of the pulpal and trabecular veins.

In the liver the pigment was present in many Kupffer cells but rarely in hepatic cells. In the lung it occurred in a few alveolar phagocytes as well as in occasional connective tissue macrophages adjacent to the smooth musculature of the bronchi or in localized areas of inflammatory change.

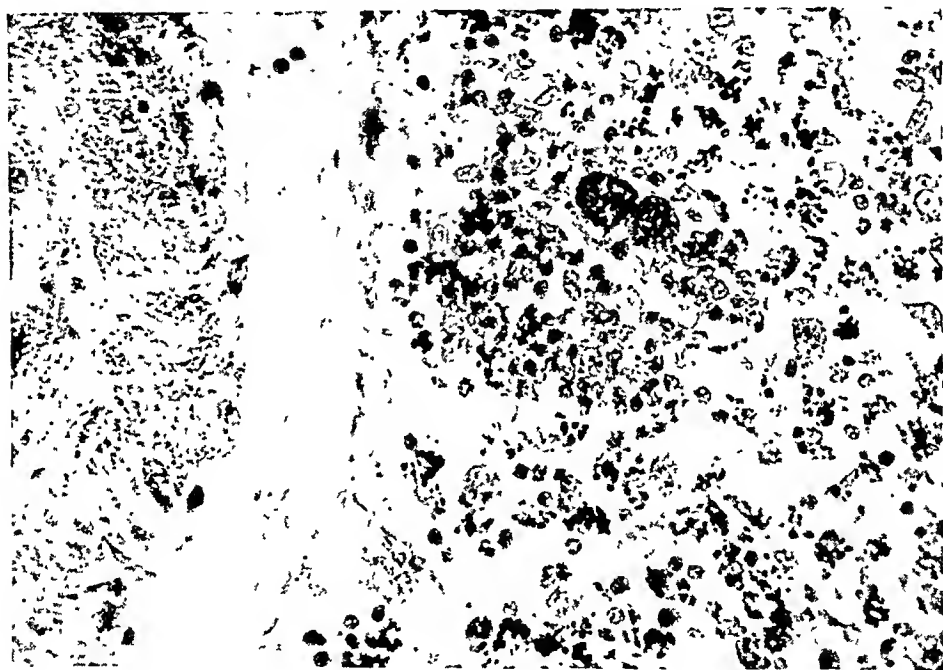


Fig. 4.—Cortex of a lymph node showing much pigment in several large fixed macrophages, in lining cells and free macrophages of the sinuses, and in the wall of a small artery just external to the capsule. Acid-fast staining; $\times 430$.

The adrenal gland showed fine granules of pigment in some cells of the zona reticularis, in macrophages bordering the sinusoids of the cortex and in a few connective tissue macrophages of the medulla. Much of this pigment, like that of the ovary, considered in the following section, may occur normally and may not be specifically related to vitamin E deficiency. Small amounts occurred in the adrenal glands of 2 control monkeys.

Sex Glands.—The testes of 1 male were quite infantile; those of the other showed only a few seminiferous tubules in which secondary spermatocytes or spermatids were differentiated. No pigment was

observed in the seminiferous tubules or in the interstitial tissue. The ducts of the epididymides were small and empty. The general picture was one of immaturity rather than one of degenerative change.

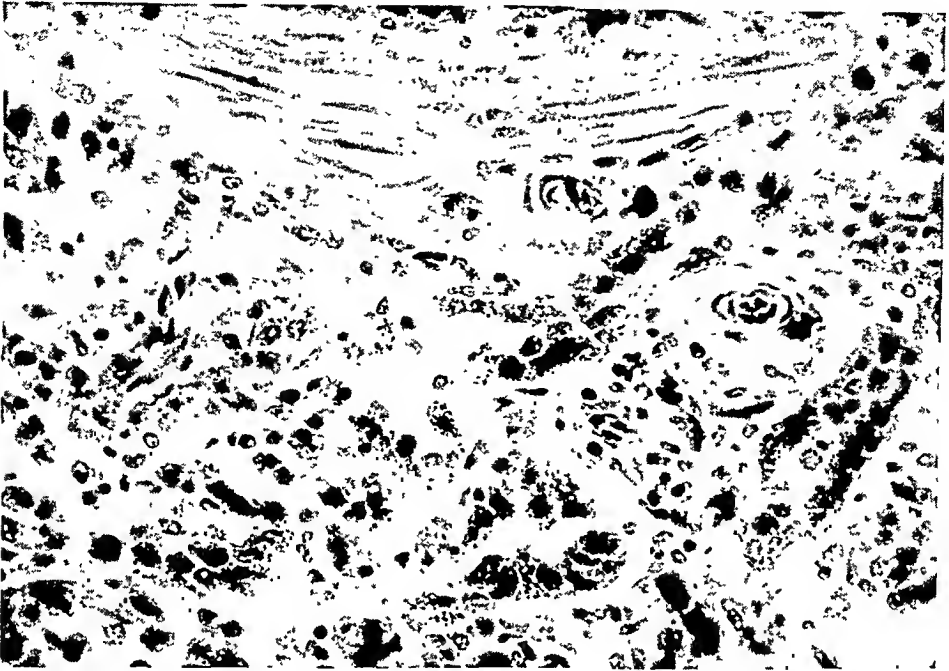


Fig. 5.—Portion of spleen showing absence of pigment in the smooth muscle cells of the trabecula but extensive accumulations of this material in fixed macrophages lining the venous sinusoids and distributed throughout the splenic cords. Acid-fast staining; $\times 430$.

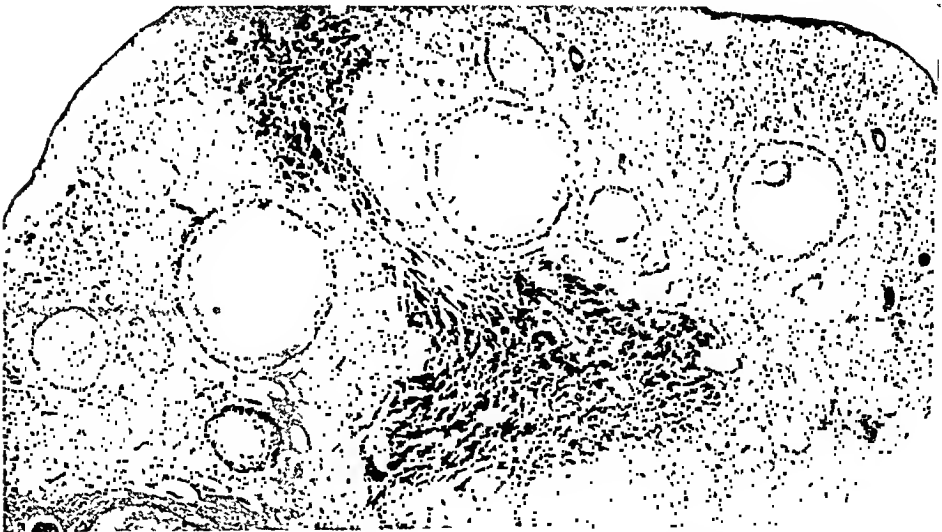


Fig. 6.—Part of ovary showing two regressing corpora lutea, composed of compact masses of lutein cells and macrophages, both containing large amounts of pigment. Acid-fast staining; $\times 30$.

The ovaries of both females contained several large corpora lutea in process of regression. These stained brilliantly with carbolfuchsin (fig. 6). The large and rather vacuolated lutein cells were uniformly filled with granules and globules of acid-fast pigment. Pigment-laden macrophages, somewhat smaller in size and less vacuolated, were present between the lutein cells and were abundant throughout the stroma of the ovary, especially in the vicinity of follicles that were in advanced stages of atresia. Only a relatively small proportion of the acid-fast material in lutein cells and macrophages showed the definite affinity for iron-hematoxylin which characterized that in smooth muscle cells. Further observations are necessary to determine how much of this ovarian pigment was due to vitamin E deficiency and how much can be considered normal in occurrence.

The ovaries of our control monkeys, because of sexual immaturity, contained no corpora lutea; moreover, they contained no cells with acid-fast pigment. On the other hand, Rossman⁵ has observed in regressing corpora lutea and macrophages in the ovaries of normal monkeys a pigment giving a positive Feulgen reaction and staining with sudan III in paraffin sections; he has given the designation "lipolutein" to this material. Through the courtesy of Dr. George Corner, director of the department of embryology of Carnegie Institution of Washington, Baltimore, we have had the privilege of examining some of Rossman's histologic preparations. The ovarian picture closely resembles that seen in our material stained according to the methods used by Rossman. In the ovaries of normal rats a similar but more limited pattern of pigment distribution occurs; with vitamin E deficiency the amount of pigment deposited in the ovary increases markedly.⁶ Other observations² indicate the same to be true of other rodents. Because of the limited histologic material available, we are unable to determine with certainty whether the ovary of the vitamin E-deficient monkey responds in a similar manner. Little is known concerning the chemical nature of the acid-fast pigment arising as a result of vitamin E deficiency and that occurring in limited amounts normally in the ovary, the adrenal gland and certain epitheliums of the male and female reproductive tracts. The latter seems related in some unknown manner to lipids arising in cells of these organs during or subsequent to active production of steroid hormones. The former may represent unsaturated fatty acids that have undergone peroxidation and polymerization because of inadequacy of an important antioxidant, vitamin E, in the cytoplasm of the cells involved.⁷

5. Rossman, I.: *Contrib. Embryol.* **30**:99, 1942.

6. Mason, K. E., and Emmel, A. F.: *Yale J. Biol. & Med.* **17**:189, 1944.

7. Mason, K. E.; Dam, H., and Granados, H.: *Anat. Rec.* **94**:265, 1945.

Kidney.—Pigment was seen in a few macrophages between the renal tubules and, in 1 animal, as intracellular granules within cells of the proximal convoluted tubules. Fuchsinophil granular deposits occurred in the lumens of some secretory and collecting tubules, suggesting excretion of pigment by the kidney.

COMMENT

The present study provides evidence that at least one of the primates, the *Macaca rhesus* monkey, responds to vitamin E deprivation in much the same manner as do other mammalian species. It also emphasizes again the dysfunctions occurring in both striated and smooth muscle cells when these are deprived of vitamin E, as reflected in the intracellular accumulations of a yellowish brown fluorescent inert acid-fast pigment. That this pigmentation of tissue would not have occurred in monkeys fed the same deficient diet supplemented with vitamin E remains to be demonstrated but may be inferred from comparable observations on other laboratory animals; for a similar pattern of pigment accumulation noted in the rat^{1b} and in the hamster, the cotton rat and the mouse² is readily prevented by feeding mixed natural tocopherols. In view of the evidence that its formation is dependent on the presence of unsaturated fatty acids as well as on a deficiency of vitamin E in the diet, it is rather surprising that the diet used in the present study, containing only 4.2 per cent lard and 0.57 per cent cod liver oil as added fat, produced such extensive formation of pigment. This may be due, in part, to the much longer feeding period employed in the studies with monkeys. The observation that considerable acid-fast pigment occurs in human tissues, reported by Pappenheimer and Victor,⁸ is of particular interest in connection with the observations presented here.

The acid-fast pigment in the vitamin E-deficient rat seems to be released to macrophages from smooth muscle cells without appreciable loss of their structural integrity but from skeletal and cardiac muscle cells in the course of their disintegration.^{1b} Our findings in the monkey are in reasonable accord with these interpretations. While the skeletal muscles of the deficient monkeys showed relatively little pigment in proportion to the dystrophic changes observed, this may be related to the low content of unsaturated fatty acids in the diet or to species differences.

Brief reference should be made to the striking and unexplained differences in the pattern of distribution of this pigment, especially in the extent to which the smooth musculature of different organs is involved, in the monkey and in other species studied. For instance, pigmentation of the smooth muscle of blood vessels constitutes a striking

8. Pappenheimer, A. M., and Victor, J.: *Am. J. Path.* 22:395, 1946.

feature in the monkey and is widespread in the hamster but has been observed in only a few vessels of the rat and then only after prolonged deficiency. Similar changes of intestinal muscle are easily induced in the monkey, the hamster and the dog but are difficult to produce in the rat. On the other hand, changes of uterine smooth muscle are rather characteristic of the rat and have not been observed in other species. Accumulation of pigment in reticuloendothelial cells of the lymph nodes and spleen is common to all animal types studied but is especially marked in the monkey. It has been impossible to determine whether all of this material is carried and deposited there by macrophages of the connective tissues, or whether some of it may be absorbed directly from the lymph and blood streams or may arise through abnormal autoxidation of lipids within these cells. The possibility that the pigment of vascular smooth muscle represents material absorbed as such from the blood stream seems unlikely unless the mechanisms involved here are quite different from those prevailing in smooth muscle elsewhere. Whether these vascular changes were associated with circulatory dysfunctions sufficient in magnitude to account for the unexplained decline and death of the monkeys and whether the response of man to vitamin E depletion resembles in any way that shown by the monkey remain to be determined by further investigation.

SUMMARY

Four young monkeys that had been reared for two to two and one-half years on a vitamin E-deficient diet showed extensive accumulations of an acid-fast pigment in smooth muscle cells of the blood vessels, stomach, intestines, gallbladder, bronchi, urinary bladder and fallopian tube, in the reticuloendothelial cells of lymph nodes and spleen and in many connective tissue macrophages. There was gross and histologic evidence of dystrophic changes in skeletal muscles, with deposition of small amounts of pigment in the fibers. Cardiac muscle fibers contained some pigment but showed little or no evidence of damage. On the basis of similar findings in other laboratory animals, these observations are presented as evidence that a state of vitamin E deficiency can be induced in the monkey.

EFFECTS OF PODOPHYLLIN AND OF COLCHICINE ON NORMAL SKIN, ON CONDYLOMA ACUMINATUM AND ON VERRUCA VULGARIS

Pathologic Observations

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IN 1942 Kaplan¹ described the therapeutic effects of resin of Podophyllum N. F. (podophyllin) as applied topically to condyloma acuminatum. Subsequent reports fully confirmed his claim concerning the efficacy of this drug. That small epithelial tumors disappear temporarily or permanently under the action of caustics is an old and familiar observation, but the phenomenon of certain neoplasms responding to a resinous substance whose original use was that of a cathartic raises many intriguing problems. The mode of action of podophyllin is not known. Culp and Kaplan² suggested that the irritative action of the drug causes spasm of small vessels, resulting in ischemic necrosis and sloughing of the tumor. No evidence was offered in support of this hypothesis.

In the course of studies on the curative action of podophyllin as applied to condyloma acuminatum we have elsewhere³ presented clinical and experimental data and a brief summary of the histologic observations. It was observed that this drug produced certain cellular changes similar to those seen after the administration of colchicine.⁴ Colchicine, as well as podophyllin, was therefore used in the treatment of condyloma acuminatum. The purpose of the present communication is to present the histologic changes following the use of these drugs in greater detail than was possible in the earlier paper.

A portion of this work was done at the William Beaumont General Hospital, El Paso, Texas, while both authors were in the Army of the United States.

From the laboratory of the Illinois Masonic Hospital and the department of pathology of the University of Illinois College of Medicine, Chicago (Dr. King), and the department of dermatology of the Johns Hopkins University School of Medicine, Baltimore (Dr. Sullivan).

1. Kaplan, I. W.: New Orleans M. & S. J. **94**:388, 1942.

2. Culp, O. S., and Kaplan, I. W.: Ann. Surg. **120**:251, 1944.

3. Sullivan, M., and King, L. S.: Arch. Dermat. & Syph., to be published.

4. King, L. S., and Sullivan, M.: Science **104**:244, 1946.

REACTION OF NORMAL SKIN TO PODOPHYLLIN

As described in the previous communication, podophyllin made up in liquid petrolatum, was applied to normal skin and mucous membranes. Patch tests were applied to the skin of the forearm. In one group of 100 patients 30 per cent showed a positive reaction of the skin at the end of twenty-four hours. In another group of 100 patients 69 per cent had a positive reaction after forty-eight hours. The reactions subsided in four to seven days. In 9 cases specimens of skin were taken for biopsy, through the affected portion of the skin, at one, two, and seven days after the application of the drug.

To study the effect of podophyllin on normal mucous membrane, 6 patients scheduled for circumcision received preliminary applications of the drug twenty-four or forty-eight hours before operation. The excised foreskins were examined histologically.

Our descriptions of the reactions of normal skin and mucosa are based on these 15 cases. All stages obviously are not represented, since carefully timed intervals are feasible only when experimental animals are used. Although abundant experimental material is available to us at present, the results observed in animals reveal certain differences. Since experiments are still in progress, the results will not be drawn on in this communication but will be reported separately.

A well developed histologic reaction, appearing twenty-four hours after application of the drug and lasting about three days, reveals the following features: In many cases the surface is covered by a narrow layer of loosely woven hyperkeratotic material, not significantly increased in amount. The normal granular layer, with its flattened cells, is also narrow and is well defined. The upper prickle layer, however, may show definite changes. The cells are large and plump; intercellular bridges frequently are absent, while cell membranes are accentuated and heavily stained (fig. 1A). Numerous fine keratohyaline granules may be visible within these large, rounded cells. The cytoplasm stains rather palely, and a delicate linear reticulation is seen. Faint dark pink lines, suggestive of tonofibrils, may irregularly permeate the cells and are sometimes in a concentric circular arrangement. The intervening cytoplasm exhibits a pale color, resembling a bubbly vacuolation within a reticulum. This is probably not a true vacuolation but rather a rarefaction of cytoplasm. The delicate fibrils frequently extend to the thickened cell membrane but do not cross from cell to cell. The fine keratohyaline granules appear situated in the interstices between fibrils.

Many cells in the upper prickle layer reveal genuine vacuolation and have a large clear perinuclear halo. In such cells the nucleus is slightly shrunken and darkly stained, with chromatin compact and the nuclear pattern obscured.

In the deeper prickly cell layer there is a great variety of cell change. A characteristic cell, illustrated in figure 1 *A*, is large and pale, and definitely but faintly basophilic. The cytoplasm is composed of a promi-

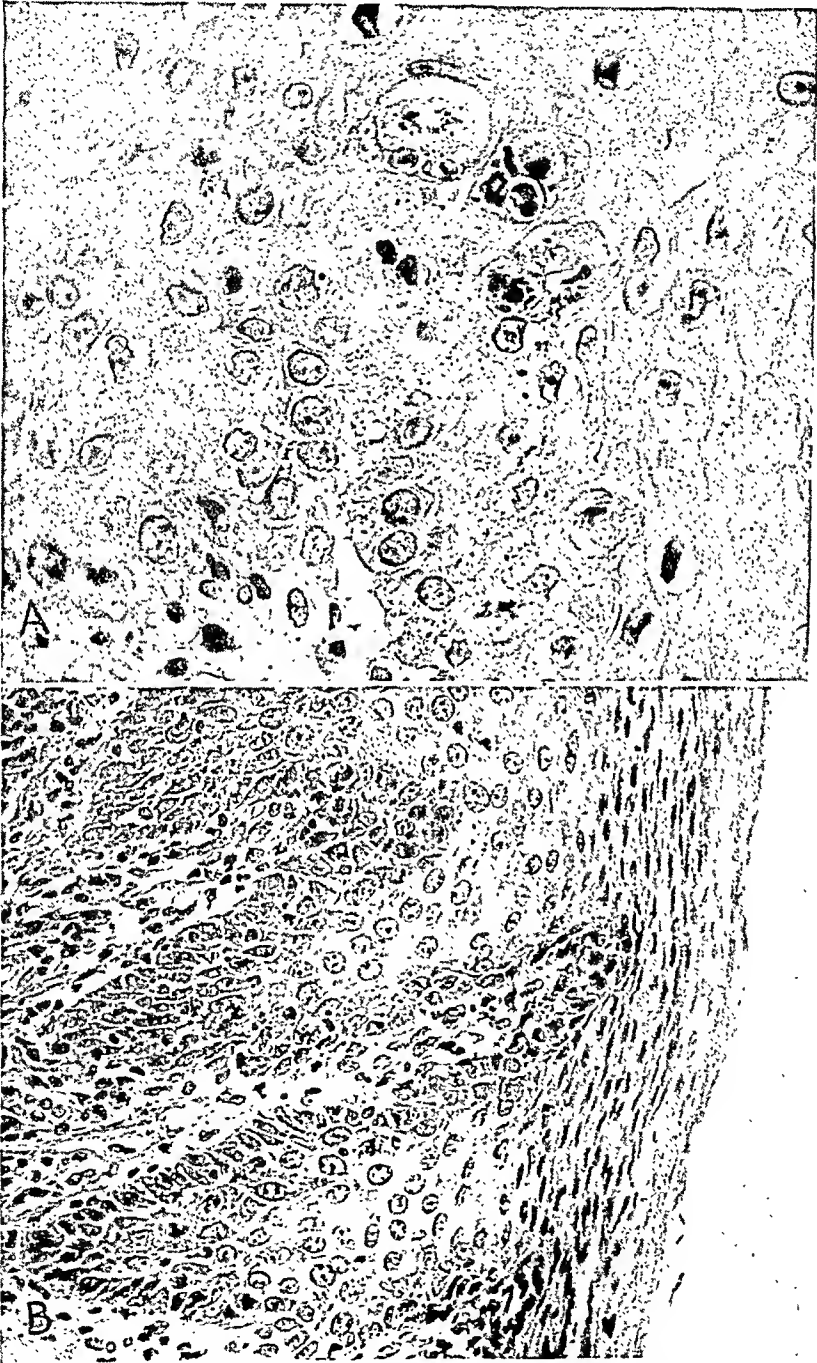


Fig. 1—*A*, effect of podophyllin applied to the skin of the forearm. Note a characteristic "podophyllin cell," surrounded by pyknotic cells. The accentuation of cytoplasmic membranes is also noteworthy.

B, prepucce twenty-four hours after application of podophyllin. Numerous distorted mitotic figures are visible near the basal layer.

ment, though faintly staining, reticulum. No nucleus is present, but nuclear material is seen as dispersed blue-staining dots or rods, which are usually symmetrically disposed within the cell, suggesting distorted mitotic figures. In some cells the entire confines are filled with reticulated cytoplasm; in others there is a variable degree of peripheral vacuolation, with central condensation of cytoplasm. The nuclear material, in the form of fine threads or granules, may be concentrated in the center or may exhibit symmetric distribution in the poles of the cells. The arrangement and the structure of the chromatin do not simulate normal mitotic phases but appear as parodies of mitosis (fig. 1 *B*).

The opposite extreme of distortion is represented by cells with bright eosinophilic cytoplasm and pyknotic or karyorrhectic nuclei. Several are seen surrounding the large pale cell in figure 1 *A*. These cells with the pyknotic nuclei show a considerable range of morphologic variation. Cytoplasm of solid texture may fill the cell confines; or peripheral vacuolation may be prominent, leaving a clear space between the cell membrane and the centrally aggregated eosinophilic cytoplasm; or the vacuolation may be irregular, with lacunas alternating with wisps of cytoplasm. The nucleus may be represented by a single solid basophilic structure, or it may take the form of several irregular karyorrhectic fragments.

Between these two extremes of cellular appearance there are many forms which can be interpreted as intermediate gradations. Thus, the nuclear material may be broken up into so many and variously disposed fragments that it is not possible to distinguish distorted mitosis from bizarre karyorrhexis. The cytoplasm may exhibit all shades of staining reaction from frank basophilia, through subtle degrees of purple, to outspoken eosinophilia. In still other variations the cytoplasm is concentrated in the center of the cell, with vacuolar spaces intervening next to the cell membrane, but with delicate strands of cytoplasm traversing the clear spaces. Nuclei preserve their general form but may be moderately shrunken, with intensification of staining and loss of clarity of nuclear detail, yet definitely not pyknotic. Cell membranes may be accentuated and thickened. The intercellular spaces surrounding such altered cells may or may not be preserved. In some instances they are exaggerated. These changes (fig. 2 *A*), most prominent in the lower prickle layer, may occasionally be found in the basal layer, while in other instances the basal layer is preserved intact. There are numerous variable alterations, consisting of changes of cell size and shape, variations of nuclear size, multiplication of nucleoli, vacuolation of cytoplasm, widening of intercellular spaces and occasional normal mitotic figures. There is no constancy in alterations of the basal layer.

In the corium there are slight edema and scanty round cell infiltration, principally perivascular. Inflammatory changes are more prominent in

sections of foreskin than in sections taken from the sites of patch tests. No vascular thrombi have been noted.

In the later stages, seven days after the initial application of podophyllin, when clinical signs of reaction have subsided, changes are relatively few. Three specimens are available for study. On the surface there is an increased amount of keratinized material, frequently of dense character, together with inconstant patches of parakeratosis. The keratohyaline granular layer is accentuated and more prominent than normal. Mild acanthosis is observed. Occasional pyknotic eosinophilic cells may be seen, of the type described in a foregoing paragraph, sometimes surrounded by whorls of well preserved cells. Some perinuclear vacuolation can be seen. Some nuclei of the basal cells may be enlarged and elongated, with a heavy chromatin content, while others are shrunken and darkly stained. Vacuolation of the basal layer is widespread. Persisting mild edema and perivascular round cell infiltration are observable in the upper part of the corium. Our series of human specimens is not sufficiently closely graded to permit following in detail the fate of the altered cells so prominent at twenty-four and forty-eight hours. It appears, however, that an essentially normal skin is reconstituted, even though at seven days faint traces of dyskeratosis and irritation can be observed.

CONDYLOMA ACUMINATUM TREATED WITH PODOPHYLLIN

Eighty patients with condyloma acuminatum were treated with podophyllin suspended in liquid petrolatum or dissolved in alcohol. Excellent results were obtained except in 3 patients with chronic, partially cornified tumors. Where clinical success was attained, involution progressed rapidly, and tumors in different stages of involution were excised. Altogether, 29 separate condylomas from 13 patients were studied, representing various phases of successful involution, and some examples of resistant nonresponsive lesions. In addition, there were 13 condylomas from 5 patients who had been treated with colchicine.

In the podophyllin-treated tumors the biopsies disclosed cytologic changes comparable to those seen in similarly treated normal skin. Figure 2 *B* illustrates the microscopic appearances present twenty-four hours after application of the drug. In the lower portion of the prickle layer are several distorted cells, whose chromatin material is broken up or clumped. The cells are sharply ballooned and swollen, with massive peripheral vacuolation and central irregular condensation of cytoplasm. A few pyknotic eosinophilic cells are present. One cell shows chromosome-like dispersion of the nuclear material. Most of the cells in the field show enlarged nuclei, with enlargement or multiplication of nucleoli and distortion of nuclear chromatin pattern. Reticulation and minor degrees of cytoplasmic vacuolation are apparent in most cells. Edema and slight leukocytic infiltration are visible in the underlying connective tissue.

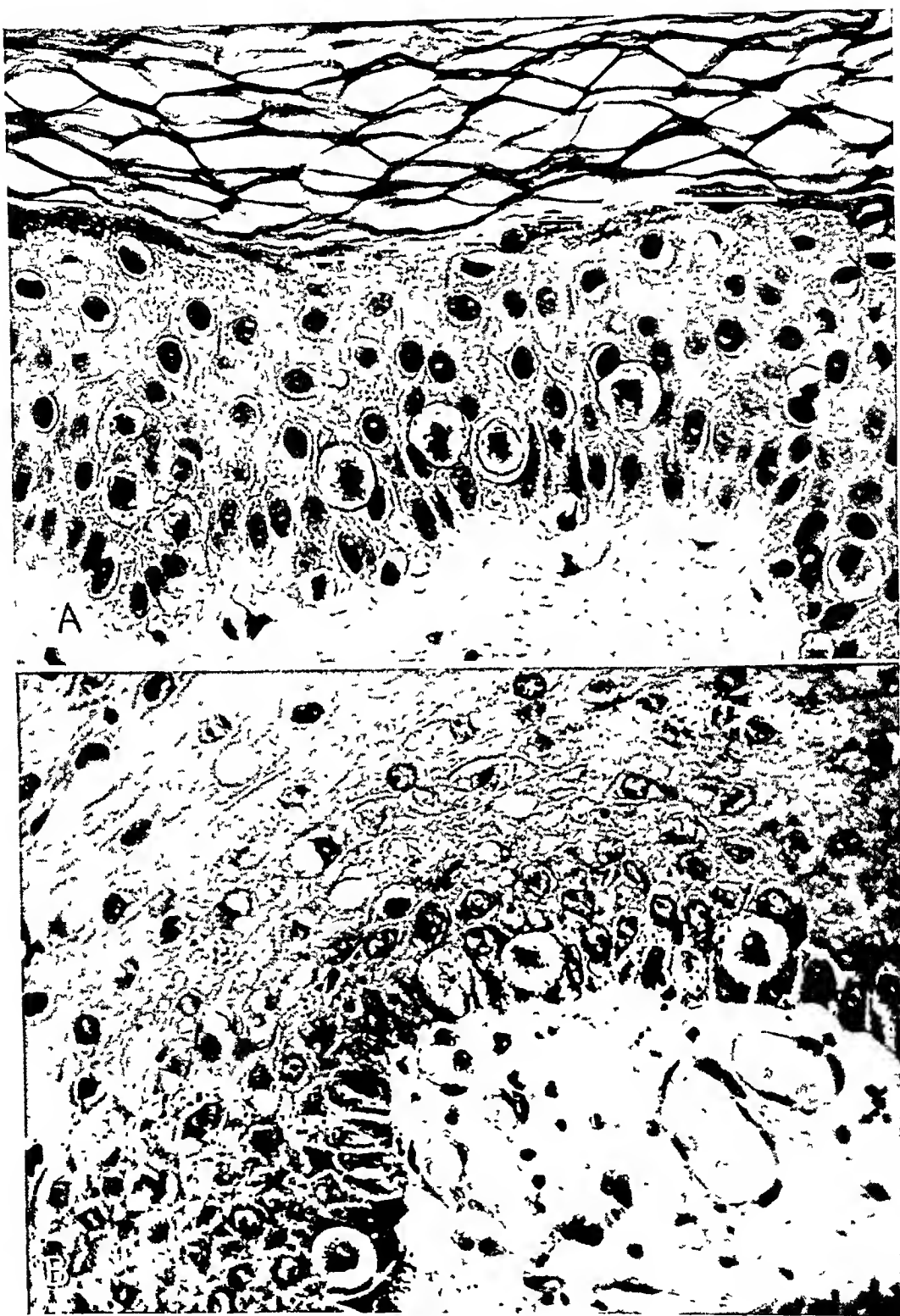


FIG. 2—*A*, different types of vacuolated and degenerating cells seen in normal skin to which podophyllin has been applied.

B, condyloma acuminatum twenty-four hours after application of podophyllin. Note the similarity to the deeper-lying vacuolated cells of *A*.

The accentuation of the cytoplasmic membranes of the more superficially lying cells is seen in figure 3 *A*, together with the loss of intercellular bridges and cytologic changes of types already described. The

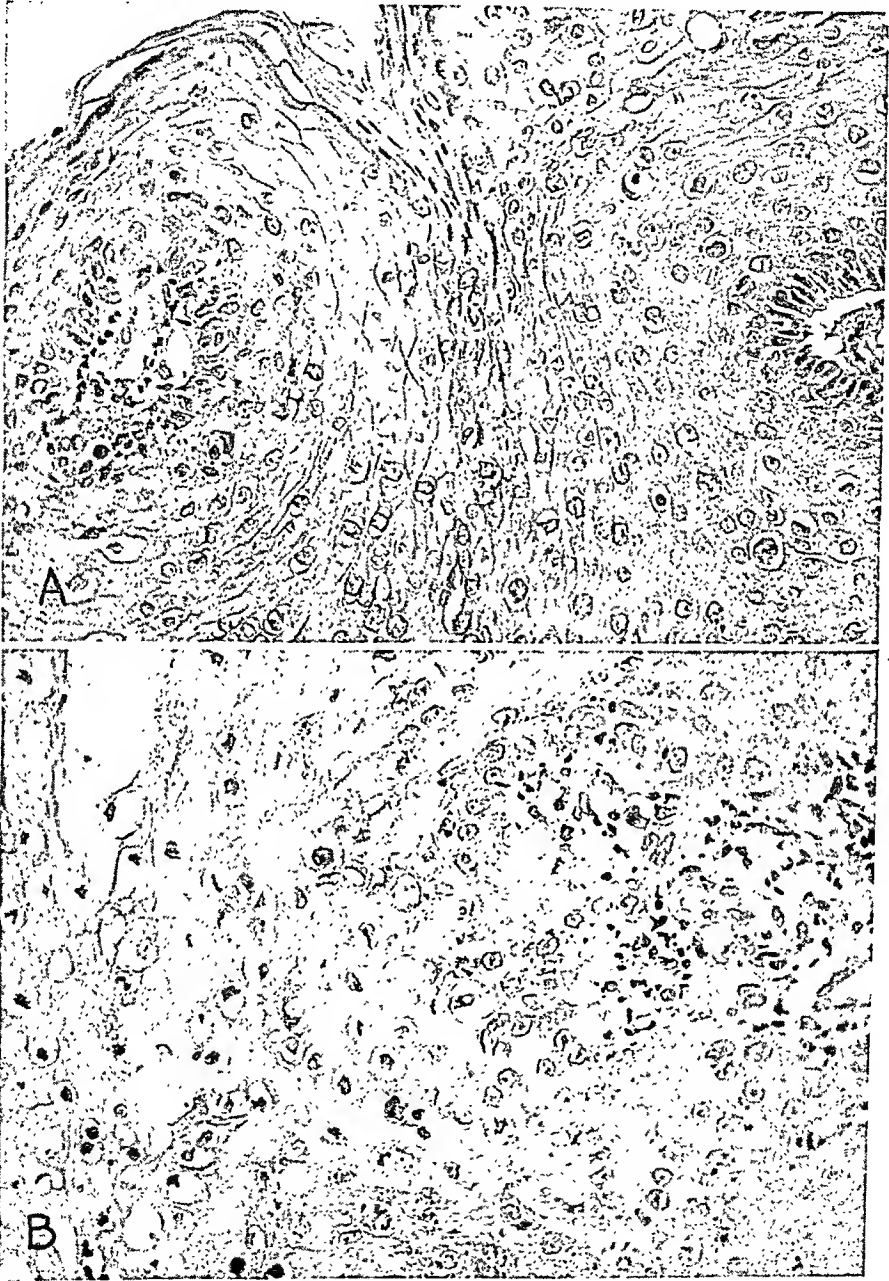


Fig. 3—*A* and *B*, condyloma twenty-four hours after a single application of podophyllin. Various forms of pyknosis, vacuolation and degeneration are prominent, as described in the text.

distortion of cell structure and the variable types of cytoplasmic vacuolation are apparent in figures 3 *A* and *B*, both of which are from twenty-four hour specimens.

Figure 4 *A* shows the residual epithelium of a condyloma which had received two applications of the resin seventy-two and forty-eight hours before biopsy. Clinically the lesion was represented only by a flat whitened patch. Histologically, virtually every cell shows severe degenerative phenomena, with shrinkage of nuclei or pyknosis and karyorrhexis, severe cytoplasmic vacuolations and marked accentuation of cell mem-

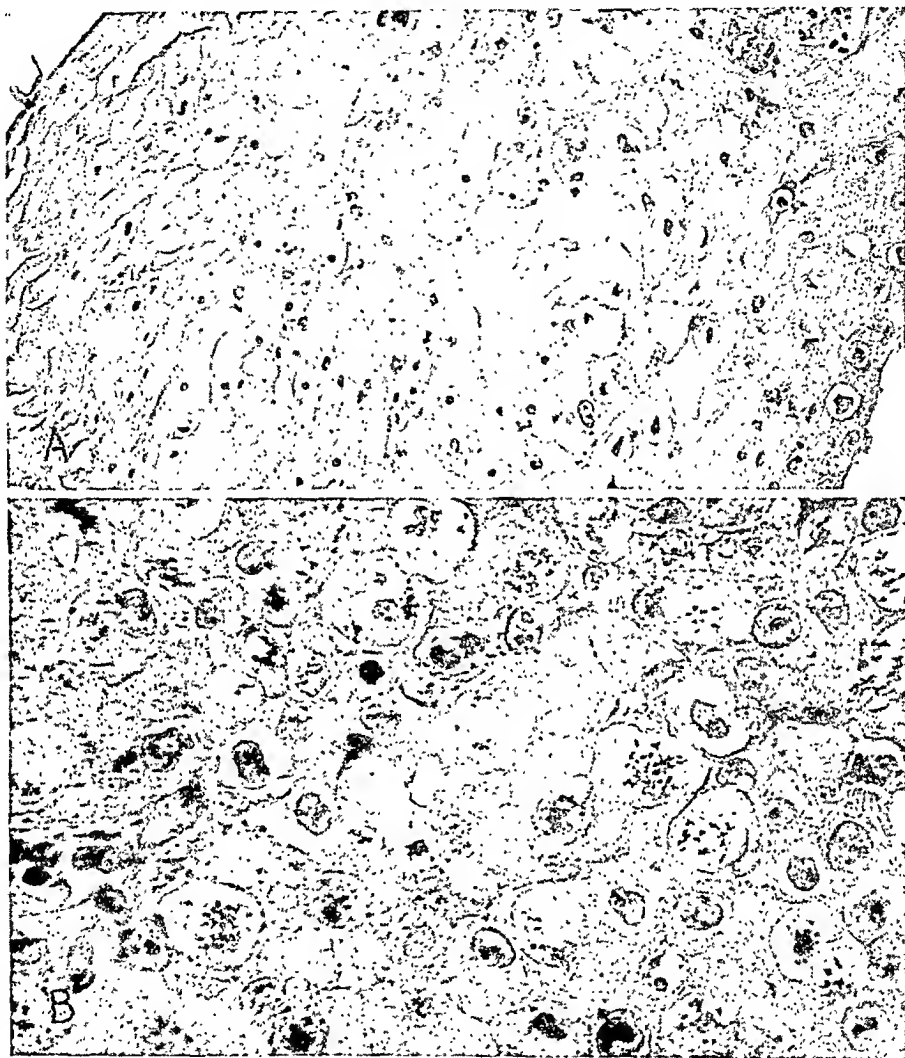


Fig. 4—*A*, condyloma showing complete involution after two applications of podophyllin. The drug had been applied seventy-two and forty-eight hours before excision. Virtually every cell reveals the degenerative changes described in the text. *B*, condyloma after three daily applications of colchicine. "Colchicine cells" are extremely numerous.

branes. Not visible in the photograph is the fine dusting with keratohyaline granules which occurs in the large pale plump cells of the midgranular layer. The so-called "podophyllin cells," illustrated in figure 1 *A*, are not in evidence. In other excised condylomas the cells in the basal and lower prickle layers are better preserved but exhibit exten-

sive nuclear swelling and distortion, enlargement of nucleoli, vacuolation of nuclei and varying degrees of hyperchromatism. A few "podophyllin cells" are occasionally present.

It should be pointed out that these curious large cells with changes resembling aborted or atypical mitoses, which we designate as "podophyllin cells" and which are illustrated in figure 1 *A*, are relatively infrequent in condylomas to which podophyllin has been applied. Cytologic changes of diverse types are widespread, but the peculiar break-up of nuclear material, so different from simple karyorrhexis in eosinophilic cells, is seen only occasionally. On the other hand, when condylomas are treated with colchicine instead of podophyllin, this type of change may be extensive. In figure 4 *B* is seen a section of a condyloma which regressed after three daily applications of colchicine and which was excised on the fourth day. Fully half of the cells of this field reveal dispersion of chromatin threads within swollen, reticulated cytoplasm. The dispersion is irregular, and under the microscope one can observe some basophilic material that has been extruded through the cytoplasmic wall.

These cells we designate "colchicine" or "podophyllin" cells, according to the agent used to produce them. In essence they are identical. The principal histologic difference between the two drugs is quantitative only. With colchicine these peculiar cells are far more numerous than they are with podophyllin. But both drugs produce abundant eosinophilic pyknotic cells, as well as others with varying degenerative nuclear and cytoplasmic changes.

Histologic study of condylomas that failed to respond clinically to applications of the drug reveals certain facts of considerable interest. In 1 patient with perianal condylomas the lesions were initially moist and pink. After applications of podophyllin they became dry and white, but they decreased only slightly in size. Representative tumors, excised three days and six days after a single application, show morphologic features approximating verruca vulgaris rather than condyloma acuminatum. There is a heavy layer of keratin, with patches of parakeratosis contained therein. The granular layer is broad and prominent, but the cells of this layer, instead of being well formed and intact, are highly vacuolated and frequently show pyknotic nuclei. The keratohyaline granules are massive and are occasionally situated within the cytoplasmic vacuoles. The prickle and basal layers exhibit no significant changes. The sections suggest that the action of podophyllin increased the cornification, instead of causing degeneration of the cells.

Two other treated tumors are in a different category. The clinical response was much slighter than was expected. One tumor received a single application of podophyllin and was excised twenty-four hours later. The second was treated with colchicine on two successive days.

and was removed forty-eight hours after the second application. In both of these, although involution was relatively slight, histologic examination revealed abundant change indicative of drug activity. Whether, if not excised, these lesions would have undergone complete involution in time is of course problematic. But that such would be the case seems entirely plausible. These two results should probably be called delayed reactions and not failures. The feature of interest is the presence of abundant cytologic change before a significant clinical effect is manifested.

VERRUCA VULGARIS TREATED WITH PODOPHYLLIN

Podophyllin and colchicine when used in the treatment of verruca vulgaris were clinically disappointing. Verrucae which had been treated with these drugs were taken for biopsy from 19 patients. The number of separate verrucae totaled 30. Specimens were excised after both single and multiple applications. For the most part, no clinical improvement whatever could be noted. In some cases the margin between skin and verruca showed some reddening, and in 1 instance there was apparent early ulceration at the margin of the verruca. Microscopic examination proves equally disappointing. The prominent hyperkeratosis and parakeratosis are completely unaffected by the drug. The broad keratohyaline layers may show cytoplasmic vacuolation but not to a greater degree than is seen in many untreated control verrucae. The prickle layer, characteristically wide, cannot be distinguished from control material, while the basal layer shows no significant alterations. Occasionally, however, the lateral edge of the verruca, where the keratin layer is thinnest, or the normal skin immediately adjacent to the lesion shows cytologic changes. "Podophyllin cells" in small numbers, together with scattered pyknotic elements, and varying types of severely vacuolated cells can be observed. The histologic changes are not qualitatively different from those already described.

The occasional reaction visible at the margin of a specimen is in consonance with the clinical observation that a few treated warts, without any shrinkage in size, became detached at their bases.

COMMENT

The cytologic effects of podophyllin are similar to those of colchicine, as has already been reported.⁴ There is an extensive literature on colchicine and related drugs, the review of which is not within the scope of this paper. Certain key contributions, however, may be cited.⁵

⁵ Ludford R. J.: Arch. f. exper. Zellforsch. **18**:411, 1936. Lits, F. J.: Arch. internat. de méd. expér. **11**:811, 1936. Dustin, A. P.: Arch. f. exper. Zellforsch. **22**:395, 1938. Brues, A. M., and Jackson, E. B.: Am. J. Cancer **30**:504, 1937. Chodkowski, K.: Protoplasma **28**:597, 1937.

Colchicine is merely one of a small group of substances which exert profound effects on cell division. In the literature these drugs have been administered by injection, with effects manifested over the entire body. Studies with tissue culture have also been made. But the present paper is concerned only with topical application of podophyllin and of colchicine to normal skin, condylomas and verrucae. Consequently, the results described in the literature cannot be uncritically adopted in the present studies, because of the difference in mode of administration. It is apparent from our studies that podophyllin can penetrate the normal skin and moist condylomas but that massive amounts of keratin such as are found in the common warts of the skin (*verruca vulgaris*) offer a barrier to its penetration and action.

The changes produced by podophyllin are of diverse types. Some cells are killed outright, as evidenced by eosinophilic shrunken cytoplasm and pyknotic or karyorrhectic nuclei. Other cells undergo morphologic disturbance which we interpret as abortive mitosis of irregular type. They are seen in relatively small numbers. Still other cells reveal a wide variety of changes which can best be interpreted as of degenerative character. In the beginning of these studies it was thought that several varieties of altered cells could be clearly isolated and carefully distinguished. Some sorts of altered cells, described in the text, occur with frequency and regularity. But so many intermediate and transitional forms were observed that we adopted the concept of a continuum of change in which the extremes are pyknosis and abortive mitosis.

A problem arises concerning the specificity of these various changes. The peculiar mitotic figures which we designate as "abortive" (produced in small numbers with podophyllin, in larger numbers with colchicine) may be considered as reasonably specific for a small group of drugs, among which colchicine has been studied most extensively. But at this point all claim for specificity must cease. Pyknosis is obviously nonspecific. The varying types of cytoplasmic vacuolation that we have described cannot be accurately classified. Two main types, perinuclear and peripheral vacuolation, occur, with gradations in between. The amount, the disposition and the tinctorial reaction of the preserved cytoplasm also vary. Along with the cytoplasmic changes, the nuclei present alterations in size and shape and in disposition and amount of chromatin, as well as nucleolar variations.

Under low power of the microscope a general pattern may be recognized, which we are accustomed to designate as "podophyllin effect." But cells of similar type can also be found, on careful examination, in occasional untreated condylomas or in tumors treated with salicylic or with trichloroacetic acid. The changes in the latter cells are not necessarily identical with the changes which we have described and illus-

trated, but a fundamental kinship is suggested. To be sure, only in the drug-treated lesions have we found such cells in considerable and significant numbers, but careful search will show rare similar cells in untreated lesions.

In the classic descriptions of *verruca vulgaris*⁶ peculiarly vacuolated cells receive mention. Waisman and Montgomery,⁷ in their study of *verruca plana*, epithelial nevus and epidermodysplasia verruciformis, described and illustrated various degenerative cell forms not dissimilar to many of the changed cells which we have observed. Sullivan and Ellis,⁸ as well as Wise and Satenstein,⁹ describing epidermodysplasia verruciformis, also comment on vacuolated forms. These alterations are by no means duplicates of what we have observed, nor do they occur in the same layers of the epidermis, but they suggest a possible relationship.

The squamous epithelial cell as it evolves from the basal layer to its final keratinization may be subjected to innumerable influences reflected in morphologic alteration. From the altered appearance we can justifiably conclude that there has been an interruption of the normal metabolic processes, but we cannot necessarily infer what that interruption has been. In the present studies, the extensive changes in the deeper layers of the epidermis we consider to be of greater significance than vacuolar or pyknotic changes in the upper layers. We infer that pathologic changes in the lower layers, where growth energy is greatest, are indicative of far greater metabolic disturbance than similar and sparser changes in the upper layers.

From our studies to date we conclude that podophyllin, as well as colchicine, locally applied, exerts a profound effect on the function of the cell, leading to extensive degenerative change. In some cells there is a stimulation leading to disorderly and irregular mitosis. This is probably also a degenerative manifestation, merely a special instance of drug action, perhaps dependent on the physiologic state of the particular cell. In condylomas the cell degeneration leads to regression of the tumor. Inflammatory changes of the corium may occur but are not directly responsible for the melting away of the tumor, and the drug's action on the cells is not mediated by vascular alterations. In just what way these drugs affect cellular metabolism there is no evidence from the present study to show.

Detailed experimental investigation is in progress and will be reported at a later date.

6. Lipschutz, B.: *Arch. f. Dermat. u. Syph.* **148**:200, 1922. Gans, O.: *Histologie der Hautkrankheiten*, Berlin, Julius Springer, 1928, vol. 2, pp. 112-121.

7. Waisman, M., and Montgomery, H.: *Arch. Dermat. & Syph.* **45**:259, 1942.

8. Sullivan, M., and Ellis, F. A.: *Arch. Dermat. & Syph.* **40**:422, 1939.

9. Wise, F., and Satenstein, D. L.: *Arch. Dermat. & Syph.* **40**:742, 1939.

SUMMARY

Podophyllin and colchicine when topically applied to squamous epithelium cause profound morphologic disturbances. The most characteristic change is the production of enlarged, swollen cells with finely reticulated, palely basophilic cytoplasm and dispersed chromatin material which shows some type of chromosomal arrangement. Other changes, more frequently seen, such as eosinophilic cells with pyknotic nuclei, various forms of cytoplasmic vacuolation and nuclear alterations, are described in detail but are not claimed to be specific. These are considered as primarily degenerative in character, reflections of the impairment of cellular metabolism produced by the immediate action of the drug. These pathologic conditions are seen in normal skin and in condyloma acuminatum when treated with podophyllin or with colchicine. The regression of the condyloma is in general correlated with the morphologic changes described. The drugs are inactive when applied to a highly keratinized structure such as verruca vulgaris.

AGE AND WEIGHT AS FACTORS IN THE DEVELOPMENT OF EXPERIMENTAL CHOLESTEROL ATHEROSCLEROSIS IN RABBITS

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VARIOUS investigators suggested that the spread and the degree of experimental cholesterol atherosclerosis of rabbits more or less parallel the degree of hypercholesteremia. The blood cholesterol level in turn depends largely on the length of time this substance had been fed to the animals. There is a limit to the quantity of pure cholesterol one can feed to a rabbit; the only restriction of the duration of the feeding experiment, however, is the life span of the rabbit, which usually is twenty-four to thirty months.

Protraction of the period of cholesterol feeding harbors fallacies one should be aware of. With advancing age, physiologic and sometimes pathologic changes develop in the rabbit which may influence the course of the experiment, its results and possibly its interpretation. Spontaneous alterations resembling atherosclerosis were observed in old rabbits on occasion.

In many a valuable report one misses information about the age and the weight of the experimental animals. These omissions render imitation of the experiments for verification or as a working basis for further research difficult if not impossible.

MATERIALS AND METHODS

In the study the weight of each rabbit was controlled daily and the dose of cholesterol fed adjusted so that 0.1 Gm. was given per 600 Gm. of body weight. Chemically pure powdered cholesterol (Merck) was weighed out on an analytic balance and filled into gelatin capsules. Size 5 capsules were used for quantities below 0.1 Gm., size 2 for amounts from 0.1 to 0.5 Gm. and size 00 for 0.5 Gm. Some rabbits had to be given two or three capsules at a time. All animals took the feeding well, none developed diarrhea, and all survived. The rabbits were fed Purina rabbit chow, and fresh vegetables were added (lettuce, turnips, spinach, carrots). The experiment was conducted from May to July. Blood cholesterol levels of all rabbits were checked by a colorimetric modification of Bloor's method at the onset of the experiment and weekly during the period of observation.

Thirty-two rabbits (1 through 32) were used in the study: One half were males (1, 3, 5, etc.) and one half were females (2, 4, 6, etc.); one half were gray and one

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This article is the third of a series (Arch. Path. 39:11 and 16, 1945).

half were albinos. Twenty-four animals were killed at the end of their feeding periods; the 8 control animals were killed at the end of the experiment. According to their age and weight, the rabbits were divided into four groups A, B, C and D. The animals in each group were comparable, being of about the same

TABLE 1.—Outline of Experiment

Group of Rabbits *	Age, Weeks	Weight, Gm.	Blood Cholesterol, Mg. per 100 Cc.	Dose of Cholesterol, Gm.	Rabbits † Fed Cholesterol the Given Number of Days						Controls After 60 Days	
					30 Days		45 Days		60 Days			
A	5	600	101	0.10	1	2	3	4	5	6	7	8
B	10	1,520	120	0.25	9	10	11	12	13	14	15	16
C	25	2,560	104	0.43	17	18	19	20	21	22	23	24
D	52	4,000	102	0.65	25	26	27	28	29	30	31	32

* These groups constituted the series used at the onset of the study.

† The serial numbers of the rabbits are given.

weight (largest variation in group D was 90 Gm.) and age. The small rabbits came from the same litters. The values for the initial weight and the initial blood cholesterol level as tabulated are average figures. A comparison of the cholesterol values of the four groups shows that the variations were slight.

CLINICAL OBSERVATIONS

The rabbits' increase of weight is evident from the upper part of figure 1. No detailed analysis seems necessary. Up to the age of 12 months the weight of a healthy rabbit is a fairly reliable measure of its age; after that the gain in weight is rather moderate. Each weight curve is a composite value for all animals in a group. There were no appreciable differences between cholesterol-fed rabbits and controls, although the weight of the control animals was slightly lower than that of the cholesterol-fed rabbits. No weight differences were observed between the two sexes or breeds of rabbits.

The blood cholesterol values of the control animals (a-d) remained stable. Cholesterol-fed rabbits reacted with a gradual and almost steady increase. The two groups of younger animals (A and B) reacted alike and so did the two groups of older rabbits (C and D). The average maximum values for the four groups were 320, 322, 340 and 367 mg. per hundred cubic centimeters of blood; the terminal blood cholesterol levels were at 320, 320, 330 and 330 mg. per hundred cubic centimeters (fig. 1, lower part). Differences between sexes or breeds were not encountered.

POSTMORTEM OBSERVATIONS

A single rabbit (30) had grossly visible atherosclerosis: Multiple elevated pale yellow patches were scattered throughout the lower part of the thoracic and all of the abdominal aorta. The largest of the plaques was 6 mm. long and 3 mm. wide. The liver of this rabbit showed steatosis, not present in any of the other animals. The initial weight of the animal was 3,985 Gm.; eight weeks later the weight was 4,360 Gm. It was the highest weight reached by any of the rabbits at termination of the experiment. The blood cholesterol of this rabbit ascended from 104 to 340 mg. per hundred cubic centimeters in the course of the study.

Sections from various organs of all rabbits were stained by different methods. Microscopically, atherosclerotic alterations were found in 4 animals (30, 29, 28, 26), all of whom were 1 year old at the onset of the experiment. The animal with

grossly visible patches (30) showed histologically the highest degree of atherosclerosis: Cross sections of the aorta at various levels revealed an almost complete ring of foam cells piled in several layers beneath the endothelium and also thickening of the intima proper (fig. 2, upper part). Another rabbit (28) had patches

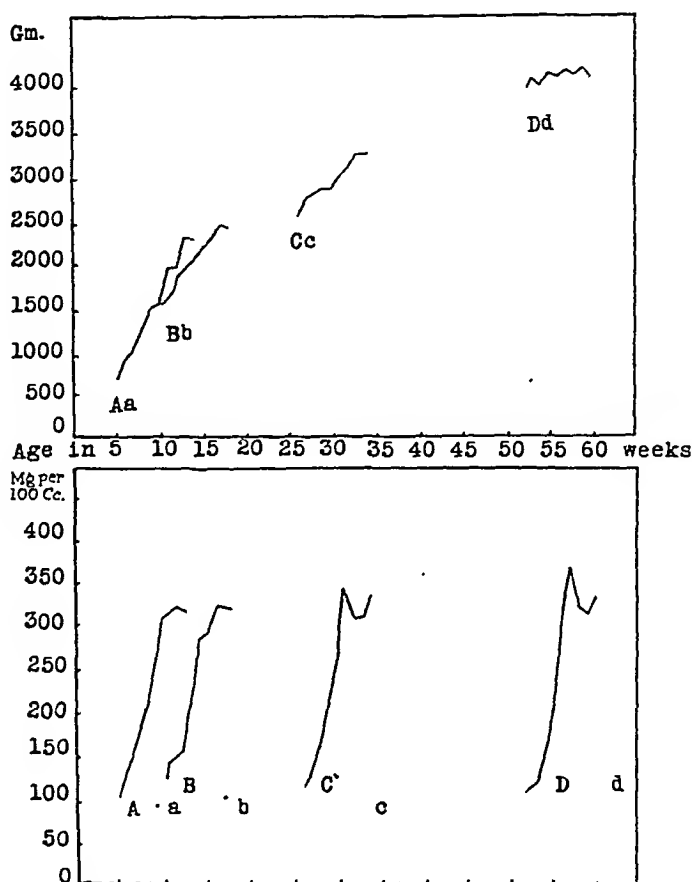


Fig. 1.—Upper part: Average weight (in grams) of the cholesterol-fed rabbits (A-D) and the controls (a-d).

Lower part: Average blood cholesterol values (in milligrams per hundred cubic centimeters) of cholesterol-fed rabbits (A-D) and of controls (a-d). The ages shown are the same for both charts.

TABLE 2.—Data on Atherosclerotic Rabbits

Rabbit	Weight, Gm.		Blood Cholesterol, Mg. per 100 Cc.			Days of Feeding
	Initial	Terminal	Initial	Maximal	Terminal	
30	3,985	4,360	104	350	340	60
29	4,040	4,200	95	340	320	60
28	4,020	4,310	112	390	300	45
26	3,950	3,970	92	280	280	30

of foam cells in the abdominal aorta only; these accumulations were less numerous and less extensive than those in the first animal (fig. 2, lower part). Finally, 2 more rabbits (29 and 26) had only isolated foam cells or small groups of up to six such cells lifting the endothelium (fig. 3). Data as to the weights and the blood cholesterol levels of these 4 rabbits are tabulated.

COMMENT

All 4 rabbits that reacted to cholesterol feeding with atherosclerotic manifestations belonged to the group of highest age and weight (D). The animal with the most severe alterations had the maximum terminal blood cholesterol value; the rabbit next in line as to degree



Fig. 2.—Upper part: Atherosclerosis after eight weeks of daily cholesterol feeding (0.1 Gm. per 600 Gm. of body weight); rabbit 30; fixation in solution of formaldehyde; rapid trichrome stain; $\times 100$.

Lower part: Atherosclerosis after six weeks of feeding; rabbit 28; fixation in solution of formaldehyde; rapid trichrome stain; $\times 100$.

of atherosclerosis was the one with the highest absolute value observed in the course of the study. While the blood cholesterol levels of the heavier animals were somewhat higher than those of the lighter

rabbits, the variation was too slight to explain the marked differences in anatomic findings. The differences were not larger than those of the initial blood cholesterol values. Also, while groups C and D had comparable blood levels, only animals of group D had atherosclerosis. Of 6 rabbits of group D, 4 responded to cholesterol feeding with

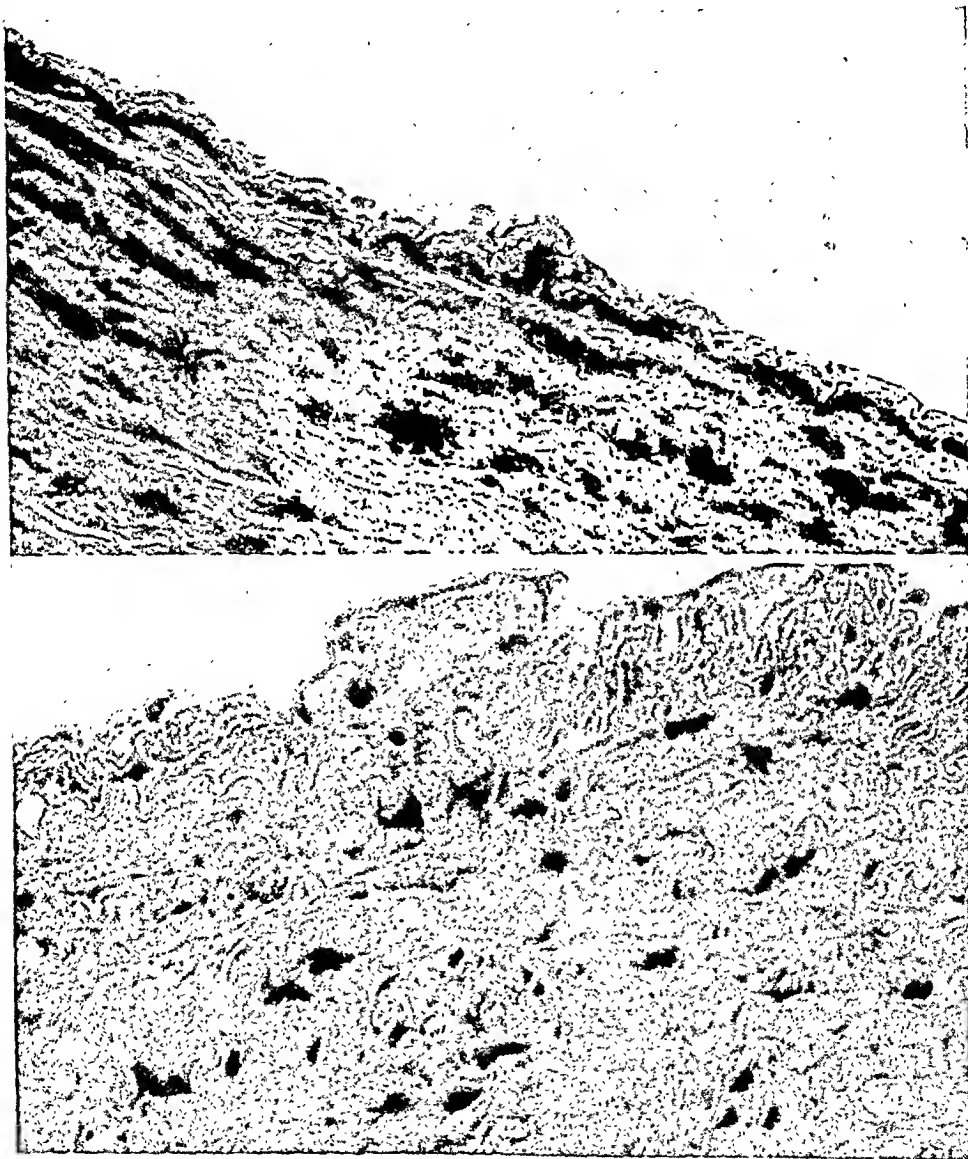


Fig. 3.—Upper part: Atherosclerosis after eight weeks of feeding; rabbit 29; fixation in solution of formaldehyde; rapid trichrome stain; $\times 475$.

Lower part: Atherosclerosis after four weeks of feeding; rabbit 26; fixation in solution of formaldehyde; rapid trichrome stain; $\times 475$.

vascular changes: 2 after sixty days, 1 each after forty-five and thirty days of feeding. The rabbit with alterations after a month of cholesterol diet had a blood cholesterol value of only 280 mg. per hundred cubic centimeters.

The study was planned so that the atherosclerotic manifestations might be detected as early as possible. Prolongation of the experiment would have been accompanied by aging of the animals. Even with our arrangement the youngest group (A) reached the weight and age of the next group (B).

Advanced age and high weight were the only points of difference between the rabbits responding to cholesterol feeding with the development of atherosclerosis and the other animals. The fact that 3 of 4 rabbits were females and only 1 a male does not seem important in view of results of previous experiments on much larger series which did not reveal any difference in the response of the sexes. The weight factor is obviously important for the development of experimental atherosclerosis of rabbits. This may apply to the age factor to some extent. By no means does it imply that atherosclerosis is a disease of old age. Experimental cholesterol-epinephrine hypertension develops more readily in rabbits after they cease to grow.¹ The individual ability to metabolize cholesterol varies: age seems to be one and weight is another of the factors responsible for such variations. The saturation point of blood cholesterol is higher in rabbits than in man. Owing to insufficient cholesterolysis, abnormal precipitation of cholesterol occurs. Atherosclerosis, then, is the result of local irritation arising from cholesterol deposits in the subendothelial layer of the arterial intima. Future efforts will be directed toward experiments in which cholesterolysis may be studied in rabbits of various age groups and weight groups.

SUMMARY

Under analogous experimental conditions, rabbits 1 year of age responded to cholesterol feeding with atherosclerotic alterations of the aorta, while rabbits 25, 10 and 5 weeks old did not. All rabbits, however, regardless of age, reacted with hypercholesteremia of almost equal degree.

Atherosclerosis of varying degree developed in 4 of the 6 rabbits that were 1 year old. The earliest manifestations were observed in a rabbit fed cholesterol for thirty days only.

It seems that the rabbit's age and weight constitute important factors in the development of experimental cholesterol atherosclerosis. Reports on experiments with rabbits should always include information concerning the age and the weight of animals.

1. Schmidtman, M., and Hüttich, M.: *Virchows Arch. f. path. Anat.* **267**: 601, 1928.

LYMPHOSARCOMA OF THE LARYNX

Report of a Case

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LYMPHOSARCOMA of the larynx is rare. MacKenty¹ reported a case of a 45 year old man with a positive Wassermann test. There was some improvement with antisyphilitic therapy, but subsequently laryngectomy was performed. MacKenty stated that:

Microscopic examination revealed marked edema and induration below the left vocal cord. The mucosa of the larynx was intact but very edematous, and in areas it showed cysts containing purulent fluid. Sections showed lymphosarcoma. Changes in the blood vessels suggesting syphilis and chronic inflammation were observed. The patient was still alive and well ten years later.

Another case was reported by Clerf² as follows:

. . . A man aged 49 years was observed during July 1934. The voice was muffled, and there was a sensation of a lump in the throat for six months. An ulcerated mass was found involving the left half of the posterior surface of the epiglottis, extending along the left aryepiglottic fold. No lymph nodes were palpable. The appearances suggested carcinoma. The report of biopsy was lymphosarcoma. . . . A total of 1,930 [roentgen] units was given to each side of the neck. The lesion disappeared promptly, and the larynx appeared practically normal. There has been no recurrence, and the patient is well.

Leroux and Petit reviewed the French literature. They found reports of 26 cases of sarcoma of the larynx. Of these, 2 were cases of lymphosarcoma.

Only 15 cases of sarcoma of the larynx of all types were reported in the English literature from 1910 to 1940.³ Additional reports have appeared recently.

Havens and Parkhill reviewed 1,100 cases of cancer of the larynx at the Mayo Clinic and found 8 cases of fibrosarcoma, 2 cases of chondrosarcoma and 1 case of rhabdomyosarcoma. However, there was not a single case of lymphosarcoma in this series.

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1. MacKenty, J. E.: Arch. Otolaryng. 20:297, 1934.

2. Clerf, L. H.: Arch. Otolaryng. 44:517, 1946.

3. Havens, F. Z., and Parkhill, E. M.: Arch. Otolaryng. 34:1113, 1941.

In 196 cases of lymphosarcoma of all types, Sugerbaker and Craver⁴ found no lymphosarcoma of the larynx. Cutler,⁵ in reporting 30 cases of generalized lymphosarcoma with 13 autopsies, noted no involvement of the larynx. Hellwig⁶ reported 202 cases of malignant lymphoma with 30 autopsies without any evidence of involvement of the larynx.

The case presented here is of interest because it is the only reported case with associated lymphatic leukemia. The incidence of lymphatic leukemia in cases of lymphosarcoma⁴ is 6.6 per cent.

REPORT OF CASE

Mrs. M. F., aged 72, was first seen Dec. 3, 1946, at which time she was complaining of hoarseness of one month's duration. There had been no loss of weight and no other complaints. Biopsy of the larynx had been made on November 19. At the time of our first indirect laryngeal examination the appearance of the lesion was recorded as follows: ". . . there can be seen a verrucous lesion, probably beginning on the right false cord and lower ventricular cavity and extending onto the true vocal cord . . ." Grossly, the lesion appeared like carcinoma. There was bilateral soft cervical adenopathy, with one firmer, slightly fixed gland just lateral to the wing of the thyroid cartilage on the left side. Also present were soft, freely movable small nodes in both axillas and both inguinal regions. The rest of the examination gave essentially negative results. A roentgenogram of the chest showed nothing abnormal.

The biopsy of the larynx in sections stained with hematoxylin and eosin showed diffuse infiltration of the submucosa with small round cells containing hyperchromatic vesicular nuclei. There was infiltration of the walls of the blood vessels. Scattered mitotic figures were seen. December 13 a biopsy was made of an inguinal lymph node, which showed diffusely arranged round or oval cells with relatively large vesicular nuclei. There was no structural pattern characteristic of lymph nodes, and there were no giant cells, tubercles or granulomatous foci.

December 16, smears of the marrow taken by sternal puncture showed cells resembling lymphocytes with dense clumped chromatin in the nuclei and narrow margins of cytoplasm. These observations were consistent with chronic lymphatic leukemia. On December 16 the white cell count was 23,100. The differential count showed small lymphocytes 72, segmented polymorphonuclear leukocytes 21, stab forms 4, monocytes 2 and myelocytes 1 per cent. The lymphocytes were predominantly immature, and a few blasts were present. The red cell count was 3,810,000; the hemoglobin content, 12.79 Gm. or 76.2 per cent (photoelectric method); the color index, the reticulocyte percentage, 2.7. The coagulation time was three minutes fifteen seconds (venous); the bleeding time, left ear two and right ear three minutes. There was partial clot retraction in three hours; the mean corpuscular volume was 100 cubic microns (normal for adults, 82 to 92 cubic microns). The mean corpuscular hemoglobin was 33.4 micromicrograms (normal for adults, 29 to 31 micromicrograms). The hematocrit reading was 38 (normal for women, 42).

In spite of the fact that this was obviously a case of generalized lymphosarcoma with lymphatic leukemia, the only symptoms were

4. Sugerbaker, E. D., and Craver, L. F.: *J. A. M. A.* **115**:17 and 112, 1940.

5. Cutler, M.: *Arch. Surg.* **30**:405, 1935.

6. Hellwig, A.: *Am. J. Clin. Path.* **16**:564, 1946.

referable to the larynx. A total of 65,000 milligram hours of radium pack therapy was given to each side of the neck, with complete regression of the lesion and marked symptomatic relief. It is emphasized that the treatment was given as palliative and symptomatic. Because of the generalized nature of the disease and the associated lymphatic leukemia, the ultimate prognosis is poor.

SUMMARY

A review of the literature reveals reports of 4 cases of lymphosarcoma of the larynx. A case of generalized lymphosarcoma with localization in the larynx has been recorded in this paper. It is the only reported case of lymphosarcoma of the larynx with concomitant lymphatic leukemia. The lesion in the larynx showed complete regression and relief of symptoms following radium pack therapy.

Case Reports

PRIMARY PULMONARY ANTHRAX WITH SEPTICEMIA

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THIS report of an unusual case of pulmonary anthrax with septicemia is presented to call attention to a disease which has all too frequently been considered to be limited almost solely to the leather and hide industries and to frankly agricultural regions.

A review of the literature showed that up to recently pulmonary anthrax was extremely rare in the United States. However, a survey from 1939 to 1943 by the United States Public Health Service¹ showed numerous cases of internal anthrax. Over the last ten years there has been only one detailed report of a case of primary internal anthrax.² Anthrax septicemia following a cutaneous lesion is not uncommon. Lucchesi³ reported a case of external and internal (pulmonary) anthrax with bacteremia, with recovery. Eurich⁴ reported from Great Britain a series of 340 cases of anthrax, in 24 of which the disease was both pulmonary and intestinal.

REPORT OF A CASE

A 46 year old white man was hospitalized with a chief complaint of cough and dyspnea of three days' duration. He had been perfectly well until four days prior to admission, when he complained of a mild cough and a feeling of general malaise. Although he did not go to bed, he had fever and a headache, and an increasingly severe productive cough developed, accompanied by pain in the left side of the chest. Slight hemoptysis began before his symptoms became pronounced. The day before admission his temperature rose to 102 F., his sputum became blood streaked, and he began to breathe with more difficulty. Twelve hours later he suddenly became much worse. His breathing became labored and fast, he was cyanotic, and he began to bring up large amounts of bloody sputum. He became weak and semiconscious and then was brought to the hospital.

His past medical history was not significant.

Social History.—He had been discharged from the Army five months previously because of age. He had not been overseas. Just prior to his illness he

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1. Smyth, H. F., and Higgins, W. D.: *Am. J. Pub. Health* **35**:850, 1945.
2. MacDonald, W. D.: *New England J. Med.* **226**:949, 1942.
3. Lucchesi, P. F.: *Am. J. M. Sc.* **183**:795, 1932.
4. Eurich, F. W.: *Brit. M. J* **2**:50, 1933

was a china and glassware salesman. Before the war he had been a rug merchant and he had recently been cleaning some new rugs which he had planned to sell.

Examination.—His temperature was 97 F.; pulse rate, 140; respiratory rate, 50; blood pressure, 90 systolic and 50 diastolic.

He was an extremely cyanotic, dyspneic, orthopneic, although well nourished, man of 46, who coughed continually. From the corner of his mouth ran a brownish red sputum. He was sweating profusely. His skin was cold and clammy. He appeared moribund. The head was normal except for the severe cyanosis. The pupils were widely dilated and did not react to light. There was no nasal discharge or obstruction. The mouth disclosed a dry and coated tongue. The pharynx was not inflamed. The neck showed no lymphadenopathy. The veins were not distended. The trachea was in the midline. The thyroid gland was not enlarged. The chest showed a lag on the left, and there was dulness to percussion over the left base and in the left axilla. Over this area there was bronchial breathing with many rales. For the remainder, the lung fields were clear. The heart was not enlarged. The sounds were regular, rapid and strong. The aortic second sound was greater than the pulmonic second sound. There were no murmurs. The pulse was rapid, weak and thready. The liver and the spleen were not felt. The extremities were cyanotic and cold. No edema, clubbing or purpura was present.

The patient was put in an oxygen tent and died two hours after admission. No laboratory work was done.

Necropsy (twenty hours after death).—The body was that of a well developed and well nourished 46 year old white man. There was marked generalized post-mortem lividity. There were no scars, abrasions, palpable lymph nodes or evidence of cutaneous areas of infection.

The left pleural cavity contained about a liter of clear yellow fluid; the right, approximately 500 cc. There were numerous enlarged hemorrhagic black mediastinal and hilar lymph nodes, measuring up to 3 cm. in diameter. The pericardium contained about 75 cc. of clear fluid.

The heart weighed 340 Gm. and appeared normal.

The right and left lungs weighed 540 and 770 Gm., respectively. The right lung was reddish gray, and its pleural surface was smooth and moist. The parenchyma was deep purplish red and poorly aerated. About the hilus and extending upward toward the apex in the mediastinum was a mass of lymph nodes, which were intensely hemorrhagic. The nodes around the hilus appeared to press on and compress the lower branch of the pulmonary artery, practically occluding the lumen. Many of the nodes looked like blood clots, while others showed traces of grayish lymphoid tissue. The left lung was heavy, and the lower lobe was enlarged and firm. The pleural surface was smooth and deep reddish purple. The entire lobe had a gelatinous and homogeneous blackish red appearance. It contained no air and was friable. The upper lobe was congested and edematous.

The spleen weighed 60 Gm. The capsule was gray and wrinkled. The parenchyma was pinkish gray, congested and mushy.

The liver weighed 1,500 Gm. The capsular surface was reddish brown and showed small circumscribed bluish black areas beneath the capsule. These measured approximately 1 cm. in diameter and proved to be small cavities which contained dark clotted blood. The parenchyma was smooth and brownish red.

Both adrenal glands were small and thin, owing to an exceptionally narrow cortex. They were pale in color and well preserved.

The right kidney weighed 170 Gm. and the left 150 Gm. The cut surfaces were plum colored, owing to marked congestion. The cortex was wide and well defined. The ureters, the bladder and the prostate were normal.

The external surface of the bowel was smooth and glistening. The gastric rugae were prominent, and numerous small superficial ulcers were noted. These were confined to the lower portion of the body and pyloric vestibule. They were most numerous along the greater curvature. The lesions measured from 0.5 to 1.5 cm. in diameter. Some were flat erythematous plaques; others showed shallow central ulcerated necrotic centers, and still others showed rolled indurated edges with fairly deep ulcerated necrotic centers. All of the lesions were covered with a thick clear gelatinous exudate. Several similar lesions were found in the duodenum, jejunum and ileum. None were found in the large bowel.

The pancreas appeared normal. The brain was not examined.

Microscopic Observations.—The outstanding histologic changes were the marked frank hemorrhage, congestion and almost total absence of polymorphonuclear cells, even in the areas of liquefaction necrosis. The preponderance of mononuclear phagocytes laden with hemosiderin in all areas of infection was noteworthy. Contrary to the recognized pathologic descriptions, the blood vessels in this case were not thrombosed. Even the smallest branches were normal. No bacilli were demonstrated in the vessels in sections stained by Goodpasture's technic.

The lower part of the left lung showed a thickened pleura infiltrated with mononuclears. The bronchi were filled with detritus; the mucosa had sloughed away partially to completely. The alveolar walls were thickened, owing to capillary dilatation. The spaces were filled with blood. There was secondary atelectasis, with practically no inflammatory response. Hemosiderin and "heart failure" cells were innumerable. The right lung showed hemorrhage in the alveolar spaces, but the alveolar walls and bronchi appeared much less affected.

The aorta showed several early atheromas. The heart revealed severe myocardial degeneration and marked congestion but no hemorrhage.

The liver contained several cavernous hemangiomas and was congested throughout, with marked fatty changes.

The renal glomeruli were enlarged, owing to congestion of capillaries. There was marked tubular degeneration, and hemosiderin-laden phagocytic mononuclears were found everywhere.

Each adrenal gland showed a narrow cortex and some autolysis.

The spleen displayed complete destruction of the internal structure. There was hemorrhage throughout. Again one was impressed by hemosiderin-laden macrophages.

The stomach revealed congestion of the vessels, hemorrhage, necrosis and edema, with ulceration of the mucosa. Large rod-shaped bacilli were numerous in the ulcer craters and invaded the mucosa in great numbers, extending down to but not into the submucosa. Hemosiderin-laden macrophages were numerous.

The small intestine showed a picture practically identical with that in the stomach except that the hemorrhage was confined to the tips of the villi. Bacilli were countless around the ulcerated areas.

The pancreas showed only acute congestion and granular degeneration.

All of the hilar lymph nodes examined showed massive hemorrhage—in some cases, involving all of the node. There was complete loss of the internal structure, and in some areas, necrotic liquefaction. The nodes were filled with macrophages. The vessels were dilated and congested, but no bacilli were seen.

Bacteriologic Observations.—Aerobic and anaerobic cultures of material from the liver, the mediastinal lymph nodes and the larynx presented a moderate number

of colonies of anthrax bacilli. Staphylococci and hemolytic streptococci were isolated in small numbers from the larynx and the liver.

COMMENT

Human anthrax is not rare in the United States. The recent public health survey from 1939 to 1943 shows a 16 per cent increase of cases of anthrax for the period. The infection occurred in people who had no occupational contact with infected material. One patient was a football player (soil?). Several patients were children; one of these, 10 years old, was resident in a mining location in a state where animal anthrax had been unknown for ten years; this child died of a pulmonary and blood stream infection. Other patients were housewives. Some of these had been gardening; others contracted the disease from tooth brushes, shaving brushes and a fur coat. Many of these cases were the fatal pneumonic type.

No longer can physicians think of anthrax as a disease limited to a few industrial and agricultural areas. While Pennsylvania has had a large number of cases of anthrax annually because of its tanneries and woolen mills, the present case is a good example to prove that anthrax may occur anywhere.

The unusual picture which this case presented in the clinical findings, in the gross pathologic findings and in the histologic observations seems to warrant this report.

ACINOUS CELL CARCINOMA OF THE PANCREAS WITH EXTENSIVE FAT NECROSIS

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IN CASES of pancreatic carcinoma it is not uncommon to find small foci of fat necrosis in the pancreas or even in the neighboring peritoneum, omentum or mesentery. Fat necrosis around distant metastases has also been described.¹ The presence, however, of areas of fat necrosis in the subcutaneous tissue, the heart, the renal capsule and other localities devoid of metastases is exceptional. To my knowledge, only 1 case of pancreatic carcinoma accompanied by such widespread and distant fat necrosis has been reported in the literature.² The following report concerns a second case with similar features.

REPORT OF A CASE

A white man 56 years of age was admitted to the Hôtel-Dieu Hospital, Quebec, Canada, June 24, 1946, complaining of severe pain in both feet and hands. Dr. J. B. John kindly furnished the clinical notes.

The family and the past history were noncontributory.

Two weeks prior to admission the patient suddenly began to suffer from sharp, persistent pains in both heels. There was some local tenderness but no swelling. Later the middle finger of the right hand also became persistently painful, and for a few days the knuckle of the same finger was slightly enlarged and red. Pains also appeared in the left hand and in both elbow regions. These pains were accompanied by insomnia, fever and spells of profuse sweating. During the last two months there was a loss of 35 pounds (about 16 Kg.). The patient complained also of anorexia and periodical digestive malaise. His temperature was 101.1 F.; the pulse rate, 88; the respiratory rate, 24; the blood pressure, 110 systolic and 70 diastolic. The patient was emaciated and definitely underweight. He appeared to be acutely ill and in severe discomfort. The skin was sallow with a tint that suggested jaundice. The conjunctivas were pale. Examination of the heart, lungs and abdomen failed to show any deviation from normal. The prostate was small and presented a smooth, regular surface on rectal examination. Several areas of tenderness were found in the vicinity of the joints on both arms and legs. There were no local swellings and no limitation of joint movements.

On admission the hemoglobin content of the blood was 75 per cent; the erythrocyte count 3,350,000 per cubic millimeter, with a mean cell diameter of 7.4 microns and a color index of 1.11. The leukocyte count was 5,428 per cubic millimeter; the differential count showed segmented neutrophils 50, stab neutrophils 21, eosinophils 8 lymphocytes 19 and monocytes 2 per cent. Urinalysis gave negative

From the Institute of Pathology, Laval University, and the Hôtel-Dieu Hospital.

1. Hegler, C., and Wohlwill, F.: *Virchows Arch. f. path. Anat.* **274**:784, 1930.

2. Titone, M.: *Virchows Arch. f. path. Anat.* **277**:416, 1936.

results. The sedimentation rate was 90 mm. after one hour and 122 mm. after two hours, and the blood urea was 33 mg. per hundred cubic centimeters. Blood cultures remained sterile, and serologic tests for syphilis (Klein), Eberthella typhi. *Salmonella paratyphi*, *Salmonella schottmülleri* and *Brucella abortus* were negative.

Fourteen days after admission, July 8, a mass the size of a hen's egg appeared in the subcutaneous tissue over the anterior part of the crest of the right ilium; this mass was definitely fluctuant and tender, and the overlying skin was slightly congested. Two days later a similar but smaller nodule developed in the hypothenar region of the right hand. In the following days other nodules of various sizes appeared in the subcutaneous tissue of the back and of the hypothenar region of the left hand, of the left elbow region and at the base of the big toe of the left foot.

The sharp pains in both arms and legs persisted throughout the patient's hospitalization; they increased in severity and were little relieved by sedatives. The patient took little or no food and continued to lose weight rapidly. His temperature remained high, some days reaching 103 F.

Roentgenograms were made of the heart, aorta, lungs, thoracic wall, stomach, intestine, gallbladder and spine and of the bones and joints of the sacroiliac regions. The only abnormal findings were a moderate distention of all segments of the duodenum and a nonopacified gallbladder fourteen hours after ingestion of Priodax.

On July 18 a few cubic centimeters of brownish material was obtained by puncture of the mass in the right hypothenar region. Microscopically, this material was made up of necrotic tissue debris with only a few inflammatory, generally polymorphonuclear, cells. Bacterial examination gave negative results.

In August recurrent nausea and vomiting appeared. The liver became enlarged, extended below the right costal margin and on palpation showed a hard and nodular surface. The lower extremities became edematous. The patient died on August 18, forty-six days after admission.

Autopsy (three hours after death).—The body was that of an emaciated adult white man. The skin and conjunctivas were slightly icteric. Marked edema was present in the feet and malleolar regions. There was a noticeable swelling of the first metatarsophalangeal region of the left foot. The epidermis was intact, but in the subcutaneous layers the tissues lost their normal luster, were gray-yellowish and had the general aspect and consistence of putty. Toward the plantar region the tissues had broken down; there was a cystic cavity containing approximately 1 cc. of thick, granulated, brownish, liquid material. The joint was normal. The soft tissues of both heels showed the same necrotic transformation with many isolated and small liquefied foci. In these regions the skin was ulcerated. Similar areas of necrosis were found in the adipose tissue overlying the crest of the right ilium, in the soft tissues of both hypothenar regions, in the thenar region of the right hand, in the dorsal region of the left hand and on the external and internal aspects of both elbows. These regions were definitely swollen and covered by normal-looking skin.

The smoothly lined pleural cavities contained approximately equal amounts of clear yellow fluid: the right, 870 cc.; the left, 850 cc. In the pericardium 40 cc. of similar fluid was found. The peritoneal surfaces were dry but showed disseminated, patchy, gray-yellowish areas, waxlike in appearance and extending deeply in the retroperitoneal fat layers. The mesentery, the omentum and the intestinal serosa contained numerous irregular nodules of similar aspect.

The esophagus, the stomach and the intestine revealed no mucosal abnormalities. The tail of the pancreas was enlarged by a firm, spherical mass 5 cm. in diameter. On section this mass did not have definite boundaries and showed the same

finely lobulated structure, texture and gray-pink color as the neighboring pancreatic parenchyma. Around this mass and in the head and body of the pancreas small waxlike areas were present. The spleen weighed 200 Gm. and was normal in shape and in color; in the lower half of the internal aspect the capsule was thickened and adhered to the pancreatic tumor. The liver weighed 3,300 Gm., was increased in size and contained numerous round or oval-shaped gray-pink nodules. The largest nodule, 12 cm. in diameter, occupied the posterior region of the right lobe, had a small necrotic center and was compressing the cystic duct. The gallbladder was distended and contained a rather large quantity of clear, yellow and very fluid bile. The gross aspect of the adrenal glands was normal. Each kidney weighed 150 Gm. and was slightly congested. The ureters, the urinary bladder, the prostate and the testes were not remarkable. The fat tissue surrounding each kidney and pelvis, the bladder and the prostate showed many waxlike areas similar to those seen in the mesentery and elsewhere.

The heart weighed 310 Gm.; the endocardial surfaces, the valves and the myocardium showed no gross abnormalities; in the subepicardial fat tissue of the right and posterior aspects of the right auricle, of the right auriculoventricular groove and around the descending right coronary artery, there were many gray, cheeselike, dry, irregular areas. In all pulmonary lobes crepitation was diminished; sections revealed deep red parenchyma oozing some serosanguinous fluid; a few millimeters under the left lower lobe there was a small calcified nodule of pea size. The trachea and bronchi were congested.

The aorta was not remarkable. The inferior vena cava was distended and its lumen was filled with red thrombotic material adherent to the walls and extending from the subhepatic veins to the common iliac veins.

Chemical analysis of liquid material collected from the right hypothernar region (Dr. H. Marcoux) showed presence of neutral fats and of cholesterol in large quantities.

Microscopic Examination.—The tumor of the tail of the pancreas was made up of medium size monomorphous polygonal cells with abundant homogeneous and basophilic cytoplasm and either round or slightly indented nuclei, rich in chromatin and containing one or two prominent nucleoli. These cells were arranged in thin trabeculae, separated by strands of collagen and capillaries, and here and there were definitely oriented around minute lumens, forming thus acinous structures. Many of the cells contained highly refractile uniform granules, staining deeply with acid dyes. These granules were generally scattered throughout the cytoplasm, but in the cells bordering a lumen they formed dense clusters in the apical end and had all the characteristics of zymogen granules. Mitotic figures were exceptional. The tumor was divided into angular lobules by connective tissue septums containing blood and lymphatic vessels and small nerves. Under low power the general aspect had a striking resemblance to normal pancreatic tissue. No centroacinous cells, tubular structures or islet formations were seen. The outlines of the tumor were irregular, and the neighboring pancreatic parenchyma was distinctly infiltrated by lobulated tumor tissue. The histologic characters of the tumor were therefore those of a highly differentiated acinous cell adenocarcinoma.

Around the tumor and in the subcapsular and interlobular fat of the body and head of the pancreas, many small areas of typical fat necrosis were present.

The hepatic parenchyma showed marked stasis and a slight degree of fatty metamorphosis, but the largest part of the sections examined was made up of metastatic nodules having the same general cell pattern as the pancreatic tumor.

Most of the cells contained numerous refractile granules with all the characteristics of zymogen granules. Very small areas of the nodules were necrotic.

Most of the capillaries of the kidneys were distended by red blood cells. In a few spots the capsule was moderately thickened and a small triangular zone of

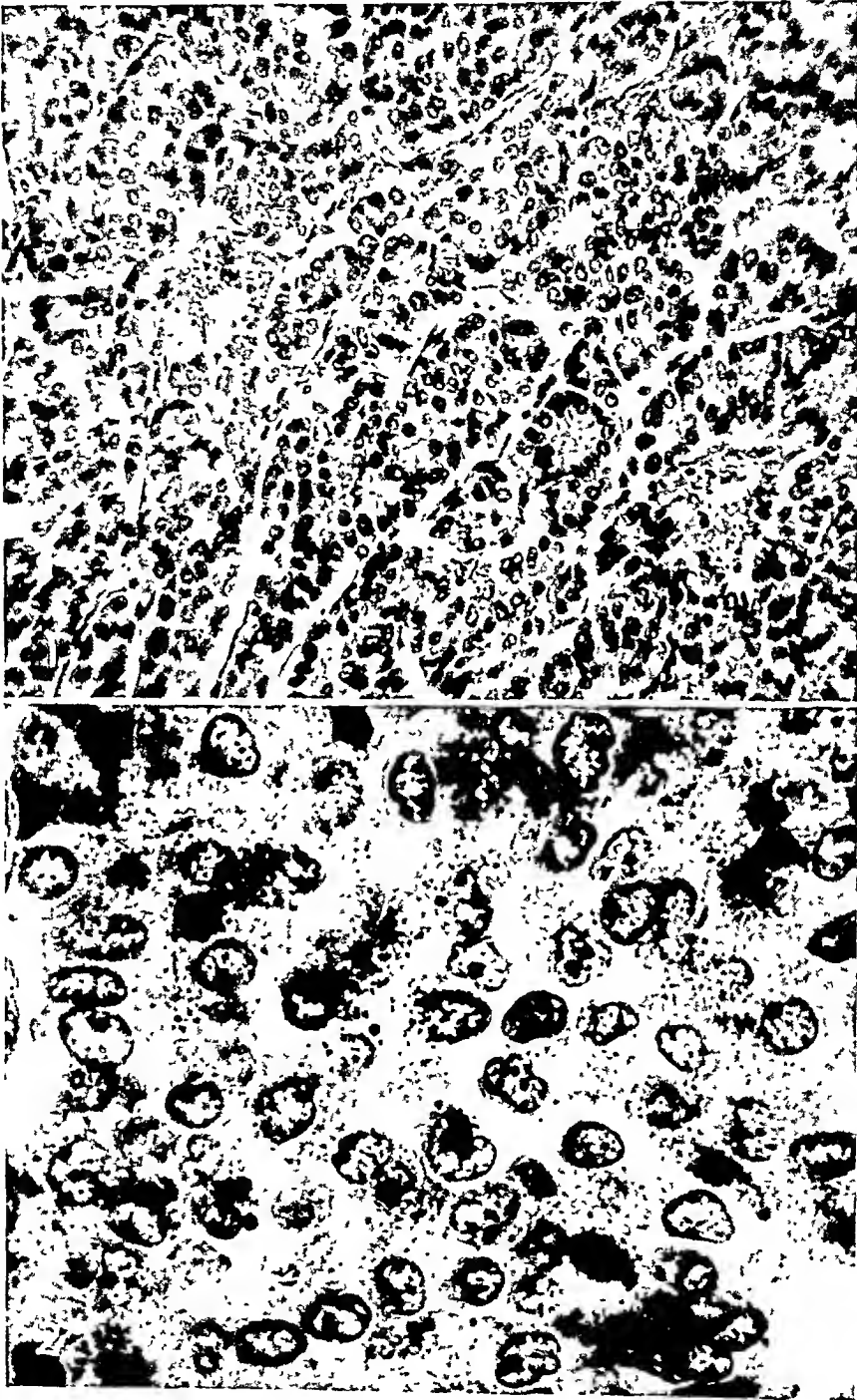


Fig. 1.—Section of the pancreatic carcinoma illustrating the resemblance to normal pancreas. Hemalum, phloxine and saffron; $\times 230$.

Fig. 2.—Section of tumor tissue showing the presence of zymogen granules. Mallory's phosphotungstic acid-hematoxylin; $\times 900$.

the subjacent parenchyma showed a moderate lymphocytic infiltrate, some tubular atrophy and a few sclerotic glomeruli. With sudan III and IV, small fat droplets could be seen in the epithelial cells of many of the distal convoluted tubules.

Sections of the mesentery, of the omentum and of the perirenal and peripelvic fat showed numerous foci of typical fat necrosis. These foci were made of cloudy fat cells devoid of nuclei with here and there small clusters of radiating crystal-like rods. Many of these foci were small, and in their center a small blood vessel with an entirely necrotic wall could be seen. The lumen of this vessel was either free or contained a small fibrinous clot. The area of necrosis was equally distributed around this vessel and appeared clearly to be oriented around it. Other much larger foci contained cystic spaces filled with necrotic cellular debris and amorphous material. Here and there thin peripheral zones of round cells were present. In none of these foci, nor in their vicinity, were any metastatic cells seen.

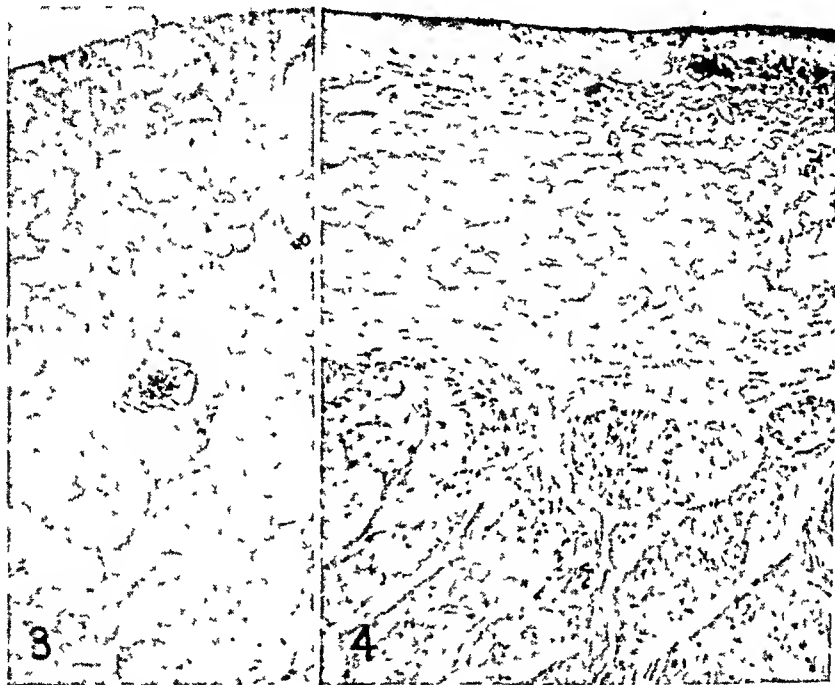


Fig. 3—Perirenal fat lobule which has undergone fat necrosis and is centered by a small necrotic blood vessel. Hemalum, phloxine and saffron, $\times 72$.

Fig. 4—Section of the right auricle showing necrosis of the fat layer of the pericardium. Hemalum, phloxine and saffron; $\times 72$.

The lumen of the inferior vena cava was occluded by a fibrinohemorrhagic thrombus without any signs of organization or fibroblastic activity. In the center of the thrombus a few linear structures were suggestive of necrotic remnants of preexisting cellular formations. In the intima of the vessels there were in one spot a few rather clear epithelial cells oriented around a small lumen. These cells, evidently undergoing degeneration, were taken as metastatic.

The myocardium and the coronary arteries showed no microscopic abnormalities. The subepicardial fat tissue, in sections of the right auricle and of the left auriculo-ventricular groove, contained diffuse and large areas of fat necrosis, partially surrounded by a small number of round cells. All the cells of these areas were more or less cloudy, and their nuclei had disappeared. The walls of the small blood vessels of these areas were also necrotic.

The subcutaneous nodules were made up of swollen subcutaneous fat tissue in different stages of necrosis. In some spots the cell outlines were still vaguely recognizable, but generally the tissue was replaced by foci of anhistic material, irregularly stained by hematein. These foci contained large clefts and cystic spaces where the material had evidently broken down and become liquefied. The subcutaneous connective tissue septums throughout the lesions and the neighboring aponeuroses and derma showed only a slight round cell infiltrate. In sections taken from the left heel, a few small purulent foci and a limited number of gram-positive cocci were also present. In these sections the overlying skin was ulcerated. Nowhere were any metastatic cells found.

COMMENT

From the pathologic observations it seems reasonable to conclude on morphologic grounds alone that in the present case the carcinoma of the pancreas and the hepatic metastases were functional. The tumor tissue was clearly of the acinous cell type and contained numerous granules, evidently zymogen granules. Functional activity of acinous cell carcinoma is not unlikely. Sugiura and associates³ compared the amylolytic action on starch, the proteolytic action on casein and peptone and the lipolytic action on several esters of extracts of a parenchymatous adenocarcinoma of the pancreas with those of three normal pancreases and found no significant differences. Comfort and associates⁴ reported a case of acinous cell carcinoma of the pancreas in which values for lipolytic and amylolytic activity in the serum and ascitic fluid were exceedingly high. These authors came to the conclusion that these high values in all probability were due to functional activity of the tumor. In the case presented here no enzyme studies were made. The extensive fat necrosis is in favor of a high lipolytic activity of the blood serum. The necrotic areas did not contain any tumor cells and appeared to have developed, in general, perivascularly. Many of these areas were plainly centered by a small necrotic blood vessel. The absence of centroacinous cells and tubular structures in the tumor tissue is also in support of the conclusion that the cellular secretion is taken up by the blood stream. This case is reported as another example of how neoplasms may carry on some of the functions of their parent cells.

The course of the illness was accelerated by thrombosis of the inferior vena cava. The association of venous thrombosis, often multiple, with carcinoma of the pancreas is frequently encountered.⁵

SUMMARY

A case of highly differentiated acinous cell carcinoma of the tail of the pancreas with hepatic metastases is reported. Many of the tumor cells contained zymogen granules. Widespread fat necrosis of the subcutaneous tissue, the heart, the peritoneum, the omentum, the mesentery, the perirenal and peripelvic fat and other localities was present. These findings are in support of the conclusion that the tumor was functional.

3. Sugiura, K.; Pack, G. T., and Stewart, F. W.: *Am. J. Cancer* **26**:351, 1936.

4. Comfort, M. W.; Butt, H. R.; Baggenstoss, A. H.; Osterburg, A. E., and Priestley, J. F.: *Ann. Int. Med.* **19**:808, 1943.

5. Sproul, E. E.: *Am. J. Cancer* **34**:566, 1938.

RUPTURE OF THE HEART FROM BLAST INJURY

JOSEPH M. MILLER, M.D., FORT HOWARD, MD.

THE USE of powerful explosives during World War II has served to focus the attention of the medical and surgical professions on injuries resulting from their use. The syndrome of blast injury has become well recognized, and injuries of the brain, the lungs, the cardiovascular system and the intestines have been described.

Many of the persons so injured have been battlefield casualties, and opportunity for postmortem examination has not been afforded. Conjecture only is possible in considering the cause of death in these cases. However, rather unusual pathologic conditions were found at postmortem examination in a soldier who had suffered a severe blast injury. It may be that the acute cardiac tamponade which he suffered may not be as rare as is commonly thought and that more such injuries might have been found if opportunity for postmortem investigation had been afforded. Acute tamponade of the heart in civilian practice is most commonly the result of stab or gunshot wounds.

Recorded cases of acute tamponade of the heart from blast are infrequent. The patient whose history is being reported was a 19 year old white soldier, a member of a blasting and drilling crew, who was accidentally injured in a premature explosion of dynamite while he was working directly over a drill hole in which the explosive had been placed. Death apparently occurred almost immediately.

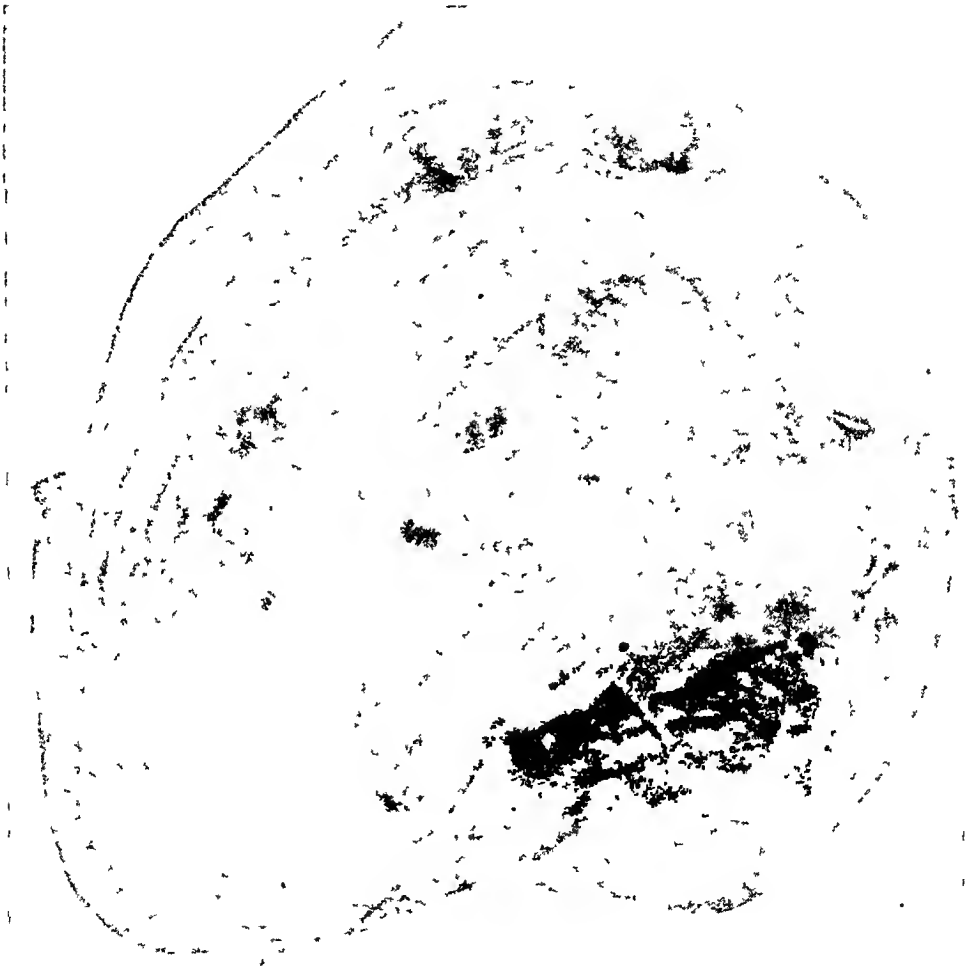
Postmortem examination revealed multiple cutaneous injuries. Several of the teeth of the upper and lower jaws were fractured at the roots.

The left pleural cavity contained about 200 cc. and the right about 60 cc. of sanguinous fluid. The lungs each weighed 675 Gm. The pleural surfaces of the lungs were smooth, moist and glistening, but the lungs were deep red, with decreased crepitation. The cut surfaces were deep red, and a sanguinous fluid was seen to ooze from them when they were cut. The bronchi were deep red and contained sanguinous fluid.

The pericardial sac was tense and blue-black, with the color of the enclosed blood showing through it. About 700 cc. of liquid and coagulated blood was found within the sac. The heart weighed 240 Gm. and measured 10.5 by 9.5 by 7 cm. Three rents were found in the left ventricle (figure) measuring 3, 2 and 1 cm. in length. The largest was located 1 cm. from the apex and 3 cm. from the anterior interventricular groove. The laceration measuring 2 cm. was adjacent to the left coronary artery at an angle of about 20 degrees, while the smallest laceration was found 1 cm. inferior to the base and 0.5 cm. distal to, and parallel with, the laceration measuring 2 cm. The lacerations measuring 3 and 1 cm. communicated with the left ventricle, but the tear measuring 2 cm. did not extend through the entire thickness of the myocardium. The leaflets of the valves were

smooth, moist and glistening. Abnormalities of the coronary arteries were not found. The left ventricular wall was 1.5 cm in thickness and the right 0.7 cm

A moderate injection of the mucosa of the stomach was present, and the duodenum was deep reddish purple, showing marked congestion. Several deep reddish black areas measuring up to 1 cm. in maximum diameter were found in the wall of the small intestine. A perforation about 0.5 cm. in diameter was present in the jejunum about 4 meters distal to the ligament of Treitz. A small



A photograph of the heart revealing the three lacerations in the myocardium of the left ventricle.

hemorrhagic area was found in the cecum. The remainder of the postmortem examination did not reveal anything unusual.

Death was obviously caused instantaneously by rupture of the left ventricle with the resultant hemopericardium and acute cardiac tamponade. This injury was the direct result of the blast as proved by the facts that there was no penetrating wound of the chest and the pericardial sac was intact.

HELMINTHIC INFECTION OF THE WALL OF THE GALLBLADDER

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WHEREAS the finding of worms in the lumen of the gallbladder is reported from time to time, helminthic infection of the wall of the gallbladder seems to be rare, especially in surgical material. In the textbooks of Kaufmann,¹ Faust,² Walters and Snell³ and in Hanser's⁴ article in the Henke-Lubarsch *Handbuch* this localization of the worms is not mentioned, and only the occurrence of *Ascaris*, *Opisthorchis felineus*, *Clonorchis sinensis* and *Fasciola hepatica* in the lumens of the bile ducts or that of the gallbladder is discussed. Stein⁵ found eggs of *Schistosoma haematobium* in the mucosa and sub-muscularis of the gallbladder of a 'Bantu man, who was killed in an accident. The eggs had provoked almost no reaction. There were only a few eosinophilic leukocytes and round cells. Stein⁵ cited Hashim⁶ as having reported 6 cases of bilharziasis of the gallbladder and Makar⁷ as reporting the observation of bilharzia in a gallbladder removed surgically—according to Stein,⁵ the first case in which this diagnosis was made in connection with the living.

In the case to be reported a worm was found in the wall of a gallbladder which had been removed for cholelithiasis and probable cholecystitis.

REPORT OF A CASE

A 28 year old woman, a native of Suriname, Netherland West Indies, was admitted with the history that since two days prior to hospitalization she had been suffering from violent pains in the right upper quadrant of the abdomen; the pain irradiated to the back and the right scapula. She had vomited several times. There had been no diarrhea. A month prior to this she had had an attack of the same nature but not so violent. Two years before admission she had been operated on for acute appendicitis, and three years before, for a right inguinal

From the Public Health Service.

1. Kaufmann, E.: *Lehrbuch der speziellen pathologischen Anatomie für Studierende und Aerzte*, ed. 10, Berlin, Walter de Gruyter & Co., 1931, vol. 1, p. 954.

2. Faust, F. C.: *Human Helminthology*, ed. 2, Philadelphia, Lea & Febiger, 1939, pp. 174, 221, 231 and 476.

3. Walters, W., and Snell, A. M.: *Diseases of the Gallbladder and Bile Ducts*, Philadelphia, W. B. Saunders Company, 1940, p. 173.

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5. Stein, H. B.: *South African M. J.* **12**:297, 1938.

6. Hashim, M.: *J. Egyptian M. A.* **14**:461, 1931; cited by Stein.⁵

7. Makar, N.: *J. Egyptian M. A.* **20**:512, 1937; cited by Stein.⁵

hermia. Ten years before, microfilarias had been found in her blood. She had never had malaria or bilharziasis.

Clinical, chemical and roentgen examinations gave the typical findings of cholelithiasis. No worm eggs, amebas, cysts or larvae of *Strongyloides stercoralis*

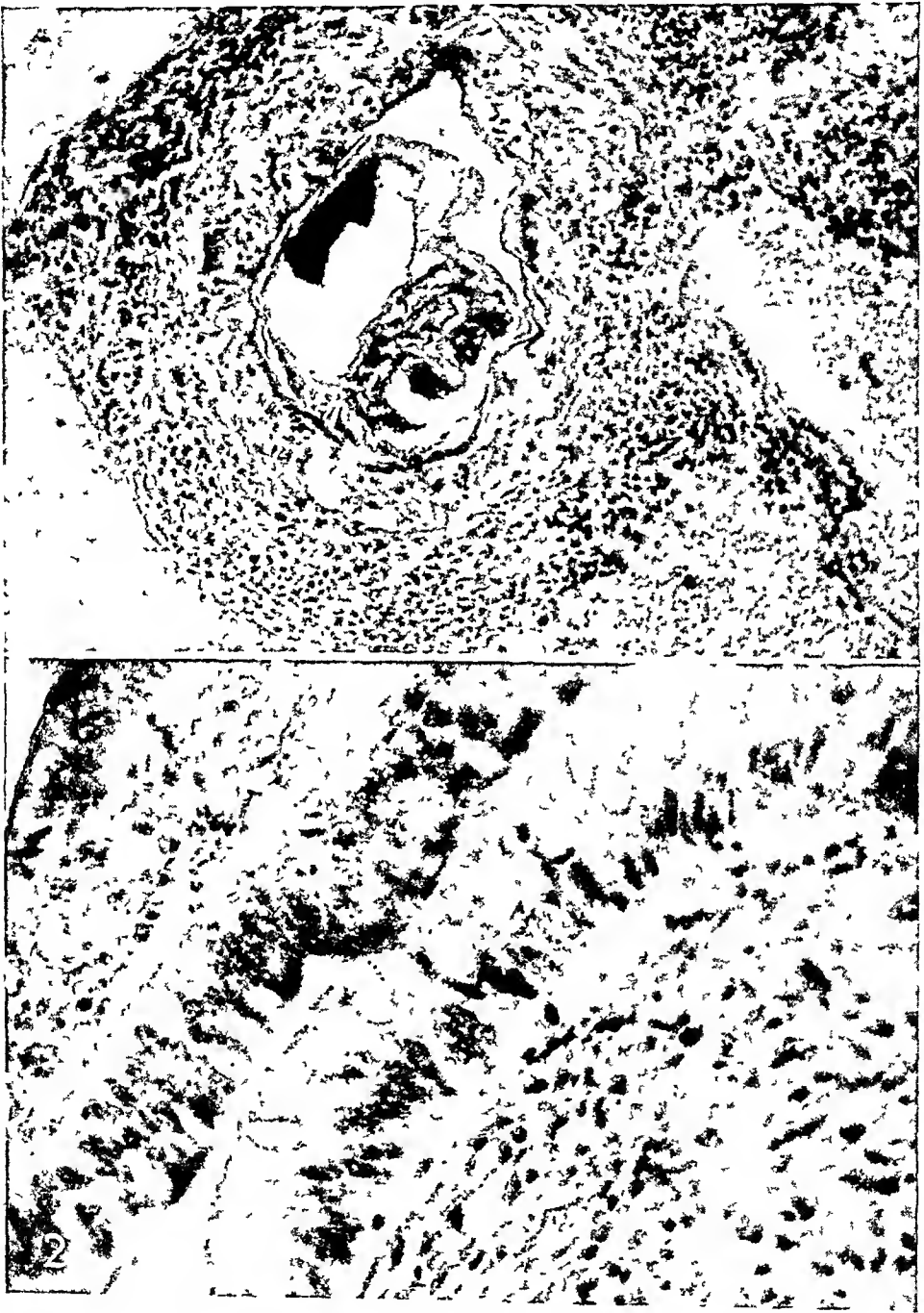


Fig. 1.—Cross section of the necrotic worm, which is surrounded by granulation tissue as it lies in a lymph vessel; $\times 190$

Fig. 2.—Goblet cells in the epithelium of the gallbladder; $\times 380$.

were found in the feces. Eosinophils constituted 21 per cent of the leukocyte count of the blood. No microfilarias were found in the blood taken at night.

Cholecystectomy was performed. The postoperative course was uneventful, and the patient left the hospital fifteen days after the operation. Three months later examination of the blood showed the presence of microfilarias.

The gallbladder was distended; the wall was slightly thickened. The viscus contained about a hundred small faceted yellowish stones. The mucous membrane was intact.

Microscopic Examination.—Immediately after its removal from the body the gallbladder was opened and fixed in the Bouin fluid-mercury bichloride mixture.^{7a} The sections were stained with hematoxylin-azophloxine^{7b} and with hematoxylin-mucicarmine.^{7c}

The surface epithelium was everywhere intact; the predominant high columnar cells showed a certain pleomorphism. Many cells had homogenous protoplasm whereas others resembled the surface epithelium of the stomach. There were also many typical goblet cells, containing distinct premucin granules. With the mucicarmine stain, cells without mucus, cells with only a little mucicarminophil substance directly above the nucleus and in the apex of the cell, cells of which the whole supranuclear region was filled with mucicarminophil substance and typical goblet cells could be demonstrated. Only the goblet cells contained premucin granules. There were many Rokitansky-Aschoff sinuses and also small glands, which normally occur only in the neck of the gallbladder but were found here also near the fundus. They opened into the Rokitansky-Aschoff sinuses. Also a few typical argentaffine cells were observed.

The epithelium, especially that in the sinuses and in the glands, but also the surface epithelium, showed marked mitotic activity. Sometimes 7 or 10 mitoses could be counted in the field of a 4 mm. objective and a $\times 7.5$ eye piece.

The muscular coat of the gallbladder was hypertrophic: the subserosa was edematous and contained markedly dilated lymph vessels, which in some places formed a kind of cavernous tissue.

In both the mucosa and the subserosa, especially in the latter, numerous eosinophilic leukocytes were found. This, together with the conspicuous dilatation of the subserosal lymph vessels made us suspect the presence of a worm, as the microscopic picture resembled that found in some cases of filariasis.

Additional sections revealed the presence of a necrotic worm in the subserosa. It was recognizable by its cuticula. In one place the probable localization of the worm in a lymph vessel could be ascertained. The greatest part of the worm was surrounded by a zone of necrosis which was walled off by elongated epithelioid cells, often containing phagocytosed material. The layer of epithelioid cells was in turn surrounded by a granulation tissue consisting of a few plasma cells, large histiocytes, often containing acidophilic material, large fibrocytes and innumerable eosinophilic leukocytes. In one section giant cells were in direct contact with the cuticula of the worm. In the epithelioid cells and histiocytes a few mitoses were observed.

The organs of the worm could not be clearly recognized. It did not contain pigment. Sometimes a red-staining substance seemed to radiate from the cuticula, resembling the clubs of Actinomyces.

COMMENT

As the worm was necrotic, it was difficult to classify it. Though the patient came from Suriname, where bilharziasis is frequent, infection

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7b. Goldner, J.: *Am. J. Path.* **14**:237, 1938.

7c. Cowdry, E. V.: *Microscopic Technique in Biology and Medicine*, Baltimore, Williams & Wilkins Company, 1943, p. 126.

with *Schistosoma* was not probable. No eggs were found in the feces or in the wall of the gallbladder. The worm did not contain pigment and was probably lying in a lymph vessel, which certainly speaks against *Schistosoma*. There were not and had never been symptoms of bilharziasis. Therefore bilharziasis or schistosomiasis must be considered as improbable.

It must be considered possible that a larva of *Ascaris* might by accident have lodged in the wall of the gallbladder. However, the worm was too large for a larval ascarid, and an adult ascarid, though frequently found in the lumen of the gallbladder and bile ducts in countries where the general rate of infection is high,⁸ would certainly not penetrate into a subserosal lymph vessel.

On the other hand, the tissue reaction was identical with the changes caused by necrotic adult forms of *Filaria* in the epididymis, and the size of the worm agreed with that of the adult of *Filaria*. It was known that the patient had been infected with *Filaria*. The examination of the blood before operation showed no filarial forms, but at a subsequent examination, unfortunately three months later, microfilarias were found. This shows at least that the patient was frequently exposed to infection with *Filaria*. In view of these facts it is certainly probable that the worm was *Filaria* and that it had gone astray and lodged in a lymph vessel of the gallbladder.

The epithelium of the gallbladder showed a marked capacity for proliferation, proved by the presence of numerous mitotic divisions. Contrary to the assertions of Maximow and Bloom⁹ and Weatherford¹⁰ many typical goblet cells were found. The changes of the epithelium certainly form a part of the pathology of the gallbladder which is almost completely neglected by the textbooks of surgical pathology and pathologic anatomy.

As the etiologic explanation of gallstones is still a matter of uncertainty, we refrain from discussing the role of the helminthic infection in the formation of the gallstones in this case; probably the stones were present before the infection occurred.

SUMMARY

The gallbladder of a 28 year old girl suffering from cholelithiasis was surgically removed. Microscopic examination revealed that the mucosa and subserosa were infiltrated with eosinophilic leukocytes, and a necrotic worm was found in a subserous lymph vessel. The worm probably was an adult form of *Filaria*. There were also interesting changes in the epithelium of the gallbladder.

8. Yang, S. C. H., and Laube, P. J.: *Ann. Surg.* **123**:299, 1946.

9. Maximow, A. A., and Bloom, W.: *A Textbook of Histology*, ed. 4, Philadelphia, W. B. Saunders Company, 1942, p. 438.

10. Weatherford, H. L.: *A Textbook of Histology*, Philadelphia, The Blakiston Company, 1944, p. 387.

TRICUSPID ATRESIA, HYPOPLASTIC TRANSPOSED AORTA AND ASSOCIATED DEFECTS OF A TRILOCULAR HEART

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THE GREAT majority of cardiac abnormalities are discussed by Abbott in her classic work.¹ Variants of recognized cardiac malformations occasionally obtain, however, and complement the entities already described. A rare combination of unusual anomalies in a functional cor triloculare biatrium with tricuspid atresia, persistent ostium primum, transposition of the arterial trunks, hypoplasia of the aorta, coarctation of the aortic isthmus and a pulmonary artery appearing to continue as the descending aorta through a widely patent ductus arteriosus is being reported. A review of the literature fails to reveal a combination of lesions identical with those in the instance being recorded.

REPORT OF A CASE

A 5 day old boy and the first of twins was admitted to the Children's Memorial Hospital, Chicago, because of dyspnea and an epigastric mass palpated twenty-four hours before admission. The infant was full term, was born as a cephalic presentation and appeared normal at birth, weighing 6 pounds and 10 ounces (3,005 Gm.). He had a meconium stool on the first and on the second day of life and was slightly jaundiced on the third day of life. A definite large epigastric mass was felt the day before admission. Respirations were rapid, irregular and grunting, and the infant looked waxy white at that time. He was put in an oxygen tent, where his respiration became more regular. Observations on the blood before admission were as follows: hemoglobin, 116 per cent; red blood cell count, 5,200,000; white blood cell count, 23,600, with the differential count showing lymphocytes 64, polymorphonuclear leukocytes 34, eosinophils 1 and transitional forms 1 per cent; 1 nucleated red blood cell was seen.

On admission the infant retained a good color apart from the administration of oxygen. There was no apparent jaundice or cyanosis, nor were there petechiae or ecchymoses. The respirations, as stated, were grunting. The lungs were clear. No murmurs could be heard. A mass, presumed to be the liver, was palpated in the epigastrium. Respiratory difficulty became increasingly evident, and despite nikethamide and oxygen the baby died three hours after admission.

Autopsy.—Except for passive congestion of an enlarged liver and patchy fetal atelectasis in lungs evidencing little congestion, the findings of note involved the heart and its great vessels.

From the Otho S. A. Sprague Memorial Institute and the Children's Memorial Hospital, Chicago.

1. Abbott, M. E.: (a) Congenital Cardiac Disease, in Osler, W.: *Modern Medicine*, revised by T. McCrae, Philadelphia, Lea & Febiger, 1927, vol. 4, p. 612; (b) *Atlas of Congenital Cardiac Disease*, New York, American Heart Association, 1936.

The heart lay in the normal position, with the apex directed to the left, and measured 71 mm. from its apex to the summit of the auricles and 35 mm. from the auriculoventricular groove to the ventricular tip. The anteroposterior diameter was 27 mm. and the maximum transverse diameter was 40 mm. A sulcus longitudinalis was not seen externally, though a rounded ventricular hump measuring 14 by 22 mm. was noted at the base of the heart along its left anterior margin. A hypoplastic arterial trunk, subsequently identified as the aorta, arose from this conus-like structure. The epicardial and the endocardial surface were smooth,



The heart has been rotated to the right to visualize the branches of the pulmonary artery and aorta. Note (1) the ventricular hump at the left anterior cardiac margin comprising the diminutive outlet chamber for (2) the hypoplastic aorta giving rise to (3) the innominate artery, (4) the left common carotid artery and (5) the left subclavian artery. The large pulmonary trunk (11) arises to the right of, and posteriorly to, the aorta and gives off the left (8) and right (9) pulmonary branches. The widened patent ductus arteriosus (12) joins the coarctated aortic isthmus (6), which continues as the aortic trunk (7). The impression is conveyed that the pulmonary artery actually continues as the aorta. Pieces of string (St) are tied to the innominate artery (3) and the left common carotid artery (4) to facilitate identification of the vessels. 10 designates the narrowed aortic arch between the left common carotid artery (4) and the left subclavian artery (5).

and the myocardium was grossly normal. The right auricle was dilated, bulging upward and to the right, and measured 36 mm. in its widest dimension, with the thickness of its wall measuring 2 mm. Dilated superior and inferior caval vessels and the coronary sinus entered the right auricle in the usual manner and clearly identified this chamber. An interauricular septal defect measuring 5 mm. in diameter opened into an enlarged left auricle, which measured 37 mm. in its widest dimension and carried a small auricular appendage. The left auricular wall measured 2 mm. in thickness. The tricuspid orifice and tricuspid valve were absent, there being nothing to mark the site of the auriculoventricular opening except some thinning at the atretic site. The left auriculoventricular opening was 9 mm. in diameter and was guarded by two distinct valve leaflets, an antero-lateral leaflet applied to the base of the common ventricle and a posterior one attached to the auriculoventricular junction at the base of the interauricular septum. The common ventricular chamber was enlarged, its hypertrophied muscle wall measuring 9 mm. in thickness. A thickening of the ventricular musculature was noted at the apex of the ventricle with some suggestion of a ridge marking the anlage of an interventricular septum. An anomalous malposed pseudoseptum composed of a hypertrophied muscle band cut off the small chamber previously mentioned as lying at the base of the common ventricular musculature along its left anterior border. An opening measuring 5 mm. in diameter afforded communication between the common ventricle and its small vestibule. The orifice of exit of the vestibule measured 8 mm. in diameter and was guarded by a tricuspid valve. The thickness of the wall of the small chamber measured 2 mm. A hypoplastic arterial trunk, identified as the aorta by the solitary coronary artery to which it gave rise, issued forth from the small chamber anterior to, and to the left of, the pulmonary artery arising from the common ventricle. The aorta measured 6 mm. in diameter and gave rise to the innominate, left common carotid and left subclavian arteries measuring 4 mm., 3 mm. and 2 mm. in diameter, respectively. The aorta narrowed appreciably after giving off the innominate artery, the aortic arch measuring 2 to 3 mm. in diameter. After giving off the subclavian artery the aortic isthmus measured only 1 mm. in diameter but was joined by an exceptionally wide ductus arteriosus, measuring 10 mm. in diameter, and continued on as the descending aorta. The pulmonary artery issuing from the common ventricle posterior to, and to the right of, the aorta had a tricuspid valve. Emerging from the ventricle, the pulmonary artery was 12 mm. in diameter. The right and left pulmonary arteries were given off from the inferior surface of this large pulmonary trunk, near the pericardial attachment, and entered the hili of their respective lungs anterior to the main bronchi. The pulmonary artery continued through the widened ductus previously mentioned to join the narrowed aortic isthmus. The aortic isthmus joining the widened ductus continued on as aorta, though the impression was conveyed that the pulmonary artery, through its exceptionally wide ductus arteriosus, actually continued as the functional aortic trunk.

COMMENT

A diminutive right ventricle is always associated with hypoplasia or atresia of the tricuspid valve or with a severe malformation of the pulmonary artery in the form of atresia or transposition.² Whether tricuspid atresia follows a failure of development of the right ventricle or represents a primary valvular abnormality is of embryologic rather

2. Taussig, H. B.: *Bull. Johns Hopkins Hosp.* 58:435, 1936.

than physiologic significance. The heart functions as a trilocular, mono-ventricular organ or as a biloculate heart, depending on the extent of the interauricular septal defect. Auricular blood empties into a common ventricle and is expelled into the systemic and pulmonary circulations. The hypoplastic outlet chamber, through which some blood is circuited, usually bears the smaller arterial trunk. Inasmuch as the ventricular structure described in the case being reported was combined with a complete transposition of the great vessels (Spitzer type IV) the aorta was hypoplastic and the pulmonary artery large, arising as it did from the main ventricle. Cyanosis was, accordingly, minimal. Indeed, complete transposition of the great vessels in tricuspid atresia is beneficial and compatible with a longer life as it facilitates pulmonary circulation. Hedinger's³ patient lived into the sixth decade and led a nonrestricted, active existence, exhibiting minimal cyanosis of the lips only on extreme exertion. A single ventricle with a diminutive outlet chamber may also occur in combination with a persistent bulbus cordis or in a more advanced stage of cardiac development in which the aorta arises from the common ventricle and the pulmonary artery from the hypoplastic chamber.⁴ In Taussig's⁴ cases both great vessels arose from the primitive outlet chamber, while in the case of Glendy, Glendy and White⁵ the ventricular and great vessel arrangements were similar to those in the case being described, although not associated with tricuspid atresia or aortic hypoplasia. A left-sided aorta arising anterior to the pulmonary artery also occurs in corrected transposition with bulboventricular inversion in which each trunk is placed in its proper ventricle.⁶

Tricuspid atresia, usually of developmental origin, has been explained as a result of overgrowth and fusion of the endocardial cushions and by an abnormal shift to the right of the interauricular septum sealing the tricuspid orifice. Obliteration of the right auriculoventricular orifice, while the commonest anomaly of the auriculoventricular cusps, is rare among the cardiac abnormalities. Abbott^{1b} found only 25 cases in 1,000 instances of congenital heart disease, 9 of which were complicated by transposition or other defects. Some degree of hypoplasia of the pulmonary artery is common, and the ductus arteriosus may accordingly be patent. Sixteen cases were of the "primary" variety associated with a patent interauricular septum and an interventricular septal defect.

Though a right-sided lesion, tricuspid atresia is manifested by left-sided enlargement in the roentgenogram and by left axis deviation in the electrocardiogram. Indeed, it is the only malformation of the cyanotic group associated with left axis deviation.² Cyanosis and dyspnea are usually present at birth in the absence of transposition, and increase in severity after the second month of life. Murmurs typical of an interventricular septal defect or a patent ductus arteriosus are inconstant and depend on the relative pressure in the systemic and pulmonary

3. Hedinger, E.: *Centralbl. f. allg. Path. u. path. Anat.* 26:529, 1915.

4. Taussig, H. B.: *J. Tech. Methods* 19:121, 1939.

5. Abbott,^{1b} p. 50, fig. 9.

6. Liebow, A. A., and McFarland, W.: *Arch. Path.* 32:356, 1941.

circulation. Hypoplasia of the right ventricle is evidenced by absence of an appreciable cardiac shadow anterior to the aorta in the left anterior oblique position and by exaggeration of the contour in the region of the pulmonary conus occupied by the diminutive chamber.⁴ While the shadow cast by transposed great vessels is usually widened in the left anterior oblique position,⁷ this would not have been the case in the instance being reported in view of the hypoplasia of the aorta and its displacement to the left. A narrow shadow might have been expected. Dilatation of the right auricle, also presystolic hepatic pulsations in the instance of a relatively intact interauricular septum bearing a small defect, are found in tricuspid atresia. Death ensues within the first year of life in the absence of transposition, though instances have been recorded of the patient's living up to 4 years of age.

* Of all the recorded cases of tricuspid atresia, only 1 case resembles, though it is not identical with, the case being reported.⁸

7. Taussig, H. B.: *Am. Heart J.* **16**:728, 1938.

8. Wason, I.: *J. Tech. Methods* **13**:106, 1934.

FATAL RELAPSING FEBRILE NONSUPPURATIVE, PANNICULITIS

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SINCE nonsuppurative nodular panniculitis was first described by Pfeifer¹ in 1892 some 30 cases have been reported in the literature, with only two or three autopsy reports.² The literature has been amply reviewed by many authors. Because of the confusing clinical picture, the obscure pathogenesis and the scarcity of postmortem reports, the case which we have observed merits publication.

REPORT OF A CASE

About June 1, 1945 a 38 year old Filipino-American corporal began to complain of cough and afternoon fever, for which he was hospitalized in the Philippines on June 8. The history was normal except for malaria in 1937 and thyroidectomy for "adenoma" in 1942. His dietary history was normal. He stated that he did not drink alcoholic beverages. Quinacrine hydrochloride, 0.1 Gm. daily, had been taken prophylactically for the preceding two years.

Except for a temperature of 101 F., examination disclosed nothing remarkable. The white blood cell count was 3,150, and the erythrocyte sedimentation rate (Wintrobe) was 20 mm. in one hour. Blood smears examined for malaria parasites, Wassermann test of the blood, urinalyses, blood cultures, and blood agglutination studies for typhoid and undulant fever gave negative results. Blunting of the right costophrenic angle was seen on roentgen examination of the chest. Examinations of sputum for acid-fast organisms showed no tubercle bacilli.

During the next two and a half weeks he had a daily fever, his temperature ranging between 101 and 104 F.; 480,000 units of penicillin was ineffective. A second white blood cell count was 2,600, while biopsy of marrow obtained by sternal puncture showed essentially normal marrow. Stools were negative for occult blood and for ova or parasites. The basal metabolic rate was + 7 per cent. The prothrombin time (Quick) was prolonged to forty-five seconds (control, twenty seconds). The serum protein was 5.8 Gm. per hundred cubic centimeters.

Between June 27 and July 8 the patient was afebrile. On July 9 his temperature rose to 100.8 F., and roentgen examination of the chest showed small areas of consolidation around the right hilus "suggesting central atypical pneu-

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1. Pfeifer, V.: *Deutsches Arch. f. klin. Med.* **50**:438, 1892.

2. (a) Miller, J. L., and Kritzler, R. A.: *Arch. Dermat. & Syph.* **47**:82, 1943. (b) Spain, D. M., and Foley, J. M.: *Am. J. Path.* **20**:783, 1944. (c) Friedman, N. B.: *Arch. Path.* **39**:42, 1945.

monia." Sputum examined for tubercle bacilli and fungi again revealed none. Treatment was supportive. Although subsequent roentgen plates of the chest became clear, the patient once more had a daily fever, his temperature rising to 103 F. On July 27 he was evacuated to the United States with a tentative diagnosis of Hodgkin's disease.

During the next four months, while he was a patient at Letterman and Barnes general hospitals,³ his chief complaints were progressive weakness and a hacking dry cough. He had almost daily elevations of temperature up to 104 F. with frequent frank chills. The physical findings were not otherwise remarkable. The lungs were clear; the heart, normal. Neither the liver nor the spleen were palpable. There was no lymphadenopathy. The skin was clear. Exhaustive laboratory studies were performed. Repeated red blood cell counts and hemoglobin determinations fluctuated between 2,500,000 and 3,100,000 per cubic millimeter and 7.5 and 9.5 Gm. per hundred cubic centimeters, respectively, with transient elevations following transfusions. Eleven white blood cell counts varied between 2,000 and 5,100. Except for a moderate shift to the left, the differential count was not remarkable. Urinalyses showed an occasional trace of albumin. A urine culture was negative. The erythrocyte sedimentation rate was 64 mm. (Westergren) and later 12 mm. (Wintrobe) in one hour. Repeated blood and stool cultures were negative, as were blood smears examined for malarial and other parasites. Blood agglutination studies for *Eberthella typhosa* H, *E. typhosa* O, *Salmonella paratyphi*, *Salmonella schottmülleri*, *Proteus* OX2, OX19 and OXK and *Bacillus tularensis* gave negative results, as did skin tests for tuberculosis and coccidioidomycosis. Examination of the blood showed chlorides 495 mg., dextrose 96 mg., nonprotein nitrogen 30 mg. and cholesterol 145 mg. per hundred cubic centimeters. The prothrombin time was seventeen seconds (control, twenty seconds). The serum protein was 5.15 Gm. per hundred cubic centimeters, with albumin 2.58 Gm. and globulin 2.57 Gm. (albumin-globulin ratio, 1). On September 4 the cephalin flocculation was 1 plus in forty-eight hours, and the icteric index was 3. Therapeutic tests with antimony, quinine, penicillin and sulfadiazine were all without benefit. He became progressively worse, and on November 16 he was transferred with condition undiagnosed to the Birmingham General Hospital, Van Nuys, Calif.

On admission into this hospital, and for the first time since the onset of his illness, a cutaneous eruption was noted. Over the flexor and extensor surfaces of both the upper and the lower extremities there were scattered discrete painless nodules measuring from 1 to 2 cm. in diameter. The overlying skin was purplish red and freely movable. The temperature was elevated to 103 F., and the patient appeared toxic and at times lethargic. His response to questioning was slow. Recent loss in weight was apparent. The eyes, the mouth, the throat and the mucous membranes were normal, as were the heart and the lungs. The edge of the liver, smooth and nontender, was felt 2 fingerbreadths below the costal margin. The spleen was not palpable, and there was no adenopathy. The red blood cell count was 3,400,000; the hemoglobin content was 11.5 Gm. per hundred cubic centimeters. The white blood cell count was 2,000, with 68 per cent neutrophils, 30 per cent lymphocytes, 1 per cent monocytes and 1 per cent basophils. Urinalysis showed only an occasional hyaline cast. Repeated determinations of the erythrocyte sedimentation rate showed 7 and 2 mm. in one hour (Wintrobe). Kahn and Wassermann tests of the blood were negative. A roent-

3. Letterman General Hospital is in San Francisco. Barnes General Hospital, closed now, was located at Vancouver Barracks, Wash.

genogram of the chest was normal. Sternal marrow showed nothing remarkable, nor were there any parasites. A nasal smear was negative for lepra bacilli.

On November 28 and again on December 5 biopsies were made of lesions of the skin. Both presented the same picture and will be described at the end of the section on the clinical course.

The patient continued to fail despite further therapeutic trials with quinine, emetine and penicillin. Supportive treatment included transfusions, parenteral

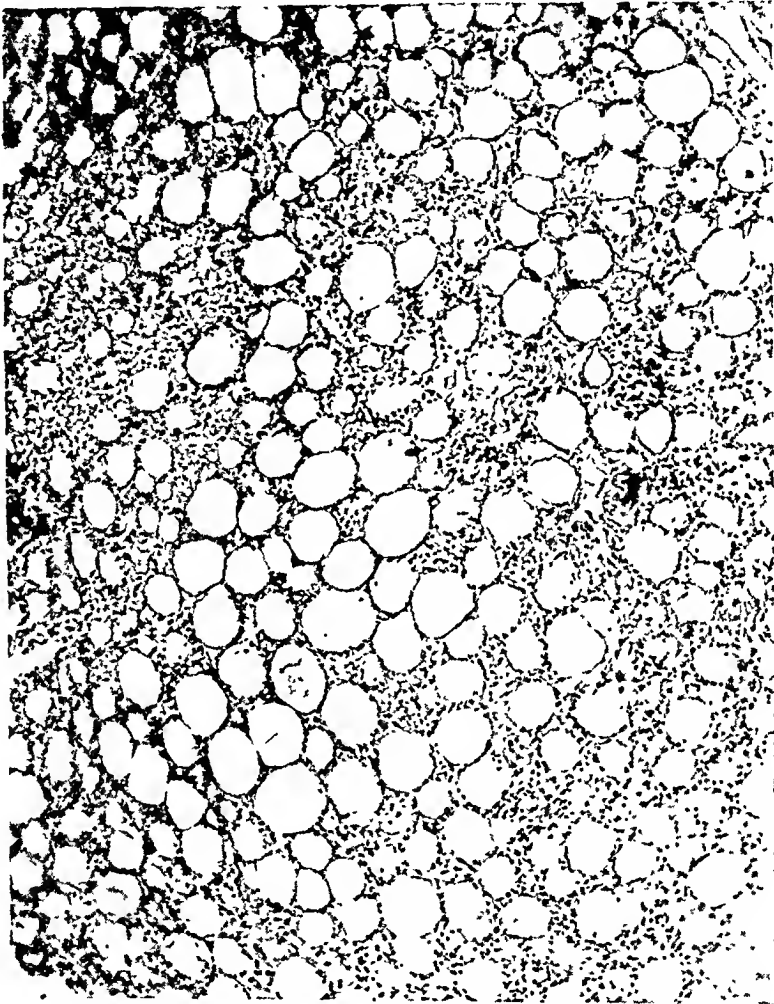


Fig. 1.—Inflammation of fat cells; low power magnification.

injection of fluids and administration of vitamins. He had a daily spiking temperature, reaching 104 F. His hacking dry cough continued. New cutaneous lesions appeared over the trunk and the forehead, while the old ones faded gradually without residual pigmentation or scarring. The liver gradually increased in size, so that shortly before death its edge, smooth and nontender, could be felt 4 fingerbreadths below the costal margin. The spleen was never palpable. There was no icterus. No angioma was seen. Repeated white blood cell counts varied between 2,000 and 5,100. Anaerobic as well as aerobic blood cultures were sterile.

On Jan. 5, 1946, seven months after the onset of his illness, the patient, while asleep, had repeated convulsions lasting several minutes. He could not be aroused thereafter, and three hours later he quietly died.

Report of Biopsy.—The biopsy specimens removed on November 28 and December 5 presented essentially similar microscopic pictures. The sections were stained with hematoxylin and eosin, Giemsa and Ziehl-Neelsen stains. These showed skin and subcutaneous tissue. The epidermis was thin and rather atrophic.

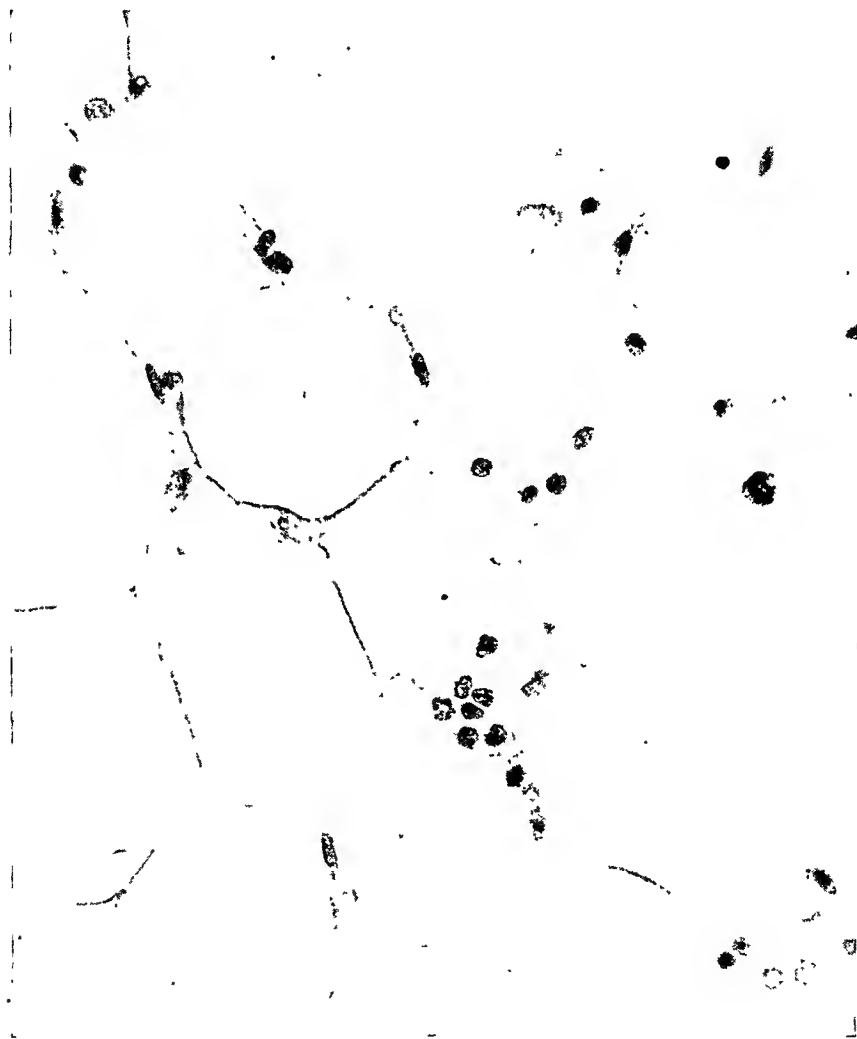


Fig. 2.—Early lesions of adipose tissue.

There was brown pigmentation of the basal layer. Some fibrosis of the dermis was seen. Around the appendages of the skin and elsewhere in the subcutaneous tissue the fat cells showed extensive involvement. The involvement, however, was not uniform. Here and there the fat cells presented an entirely normal appearance with a signet ring arrangement of the cell nucleus and an entirely clear cytoplasm. In a number of fat cells a homogeneously pink-staining material had been precipitated inside the cell membrane. The large majority of the fat cells, however, showed collapse of the cell membrane and partial or complete loss of

cellular outline. These ruptured fat cells were being invaded by varying numbers of foamy macrophages, lymphocytes, plasma cells and polymorphonuclears (figs. 1 and 2).

The inflammatory cells which had invaded the ruptured fat cells tended to arrange themselves in a row immediately within the cell membrane.

Later stages of the process were manifested by necrosis of the adipose tissue and collection of cellular exudate in what apparently constituted a damaged fat cell (fig. 3).

The macrophages were about 20 microns in diameter, had finely vacuolated pale-staining cytoplasm and large round or oval vesicular nuclei. An occasional macrophage contained one or two ingested lymphocytes and an occasional red cell. A few of the macrophages were quite large, but no multinucleated giant cells were seen. No areas of suppuration or abscess formation were found. The inflammatory reaction was occasionally more marked around small-sized blood vessels. In the superficial layers of subcutaneous tissue the inflammatory cell infiltrate was confined to the fat lobules around the appendages of the skin without involving these or the fibrous tissue. It did not extend to the dermis. Nowhere could any microorganisms be seen. There was no scarring.

The diagnosis of nonsuppurative panniculitis was made and confirmed by the Army Institute of Pathology.

Autopsy (six hours after death).—The body was that of a well developed, somewhat undernourished Filipino man, measuring 163 cm. in length and weighing about 65 Kg. There was moderate pitting edema of the lower extremities. A few ill defined, slightly depressed and lighter-colored areas measuring 1 to 2.5 cm. were scattered over the trunk and the face.

There was no icterus of the scleras. The lips were cyanotic. Both parotid glands were considerably enlarged, firm and nonfluctuant. The right parotid gland measured 3 by 3 by 1 cm.; the left, 5 by 6 by 2 cm. The glands were edematous, but no abscesses were encountered. A well healed thyroidectomy scar was present over the neck. The superficial lymph nodes were not enlarged.

The subcutaneous fat measured 1 to 2.0 cm. thick. Grossly, it showed no areas of suppuration, necrosis or nodularity. Section of the breasts revealed no abnormalities.

The peritoneum contained no fluid or adhesions. The surfaces were of normal color and luster.

The pleural cavities each contained about 200 cc. of blood-tinged fluid. The pleural surfaces were smooth and glistening.

The right lung weighed 350 Gm.; the left, 400 Gm. There was congestion, more marked on the left, where a few poorly defined, slightly bulging yellowish granular areas were present.

One hundred and fifty cubic centimeters of clear straw-colored fluid was present in the pericardial sac. The heart weighed 250 Gm. and was grossly normal. The coronary arteries, the aorta, the pulmonary artery and the venae cavae were not abnormal.

The spleen weighed 200 Gm. The cut surface presented a dark bulging parenchyma, in which the malpighian corpuscles were somewhat prominent. Thin sections were obtained with ease.

The liver weighed 2,275 Gm. It had a golden yellow color and was friable. The cut surfaces showed a bulging yellow tissue. There was no fibrosis. The hepatic artery and vein, the portal vein, the bile ducts and the gallbladder were normal.

The pancreas weighed 125 Gm. The duct of Santorini was cystically dilated and measured 1 cm. in diameter. It was filled with granular white material. The main pancreatic duct was normal. No areas of fat necrosis or fatty or fibrous replacement were seen.

Each kidney weighed 125 Gm. The cortices were pale brown and smooth. Coronal sections showed a well demarcated cortex measuring 0.7 cm. The

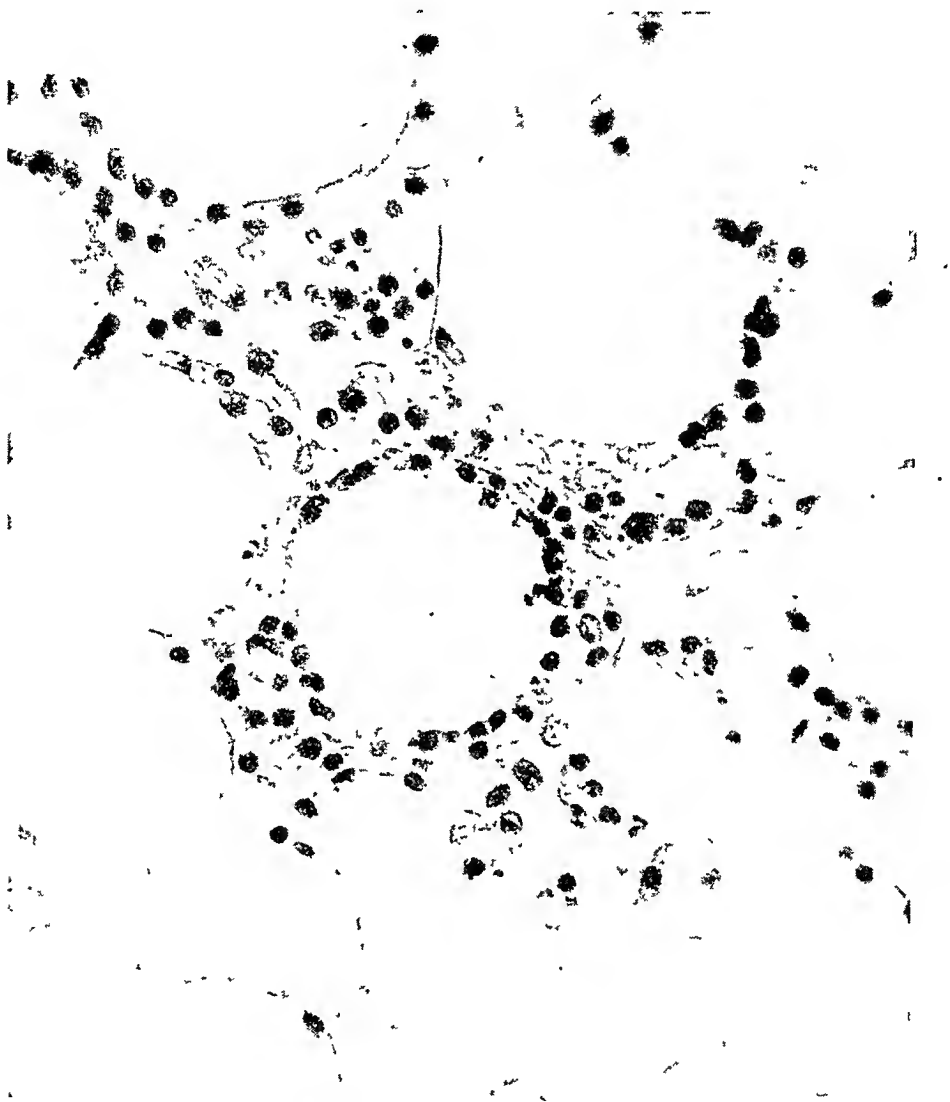


Fig. 3.—Later stages of the lesion. Note the orientation of the lymphocytes inside the fat cell membrane. Several other fat cells are being filled up with the exudate. Note the macrophages.

glomeruli were not prominent. No areas of scarring were seen. The calices, pelves, ureters and bladder and the prostate were normal.

The thyroid gland was almost completely replaced by scar tissue. The parathyroid glands were not identified. The adrenal glands and the pituitary gland were grossly normal. The testes were somewhat atrophic. The lymph nodes were not enlarged, and the cut surfaces were not particularly remarkable. The bone marrow was pale red.

The brain weighed 1,275 Gm. The leptomeninges had a gelatinous consistency. There was a pressure cone at the ventral surface of the cerebellum. The cerebral hemispheres were symmetric. Coronal sections made at 1 cm. intervals showed no abnormalities.

Microscopic Examination.—Routine sections taken from the viscera were fixed in solution of formaldehyde U. S. P. diluted 1:10, embedded in paraffin and stained

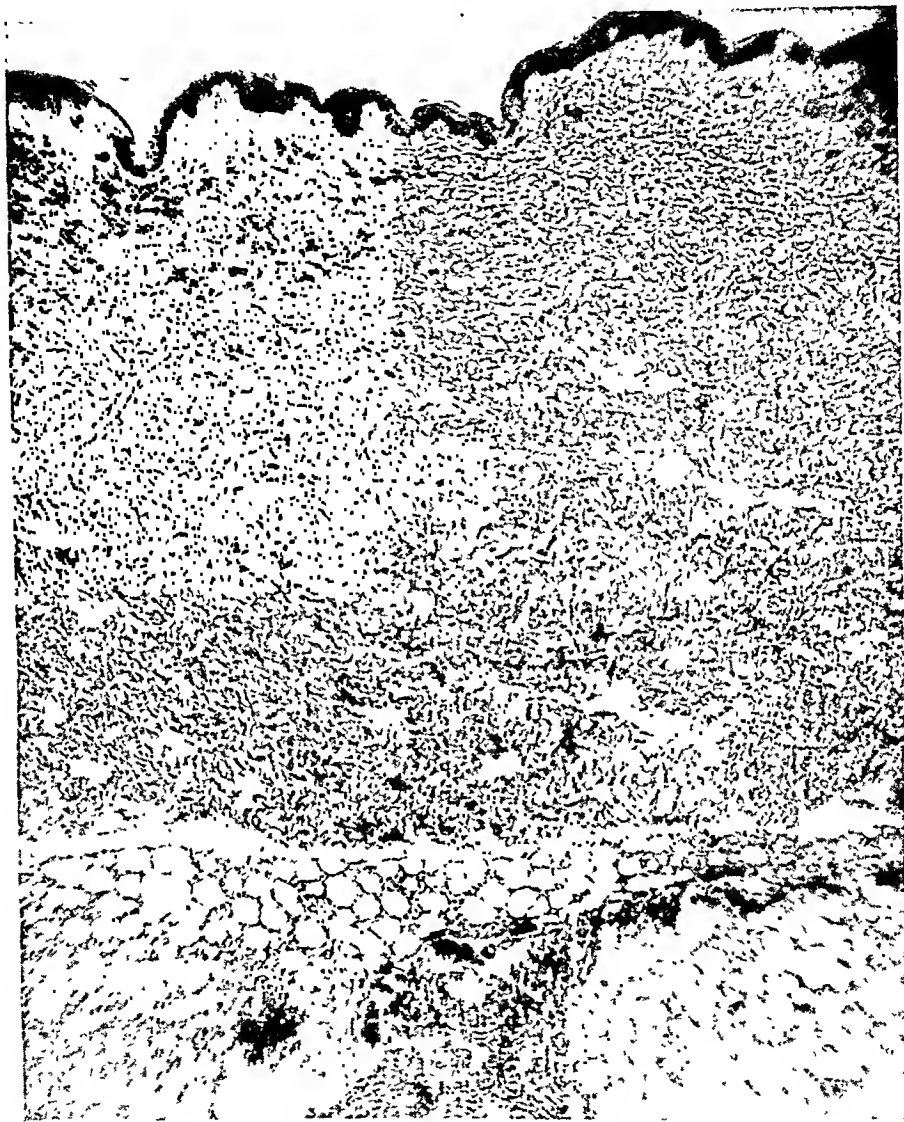


Fig. 4.—Skin and subcutaneous tissue with the inflammatory reaction restricted to the subcutaneous fat. Low power magnification.

with hematoxylin and eosin. Gram, Giemsa and scarlet red stains were prepared whenever indicated. The description will be limited to that of the pertinent organs.

Skin: The epidermis was rather thin and somewhat atrophic. There was heavy brown pigmentation of the basal layer. Some hyalinization of the dermis and subcutaneous fibrous tissue was present, but there was no inflammatory cell infiltration (fig. 4). There was considerable involvement of the subcutaneous

fat similar to that seen in the biopsy. In the deeper portions, little inflammatory cell infiltration was present, but as one approached the superficial regions one observed extensive necrosis and infiltration of the fat. No areas of suppuration or abscess formation were seen, and the inflammatory cells consisted of foamy macrophages, lymphocytes and plasma cells.

Liver: There was marked disturbance of the structure by extensive fatty change of the liver cells. The fat occurred in large and small droplets and stained bright red with scarlet red. By and large it had a peripheral distribution. Scattered throughout were areas showing varying degrees of necrosis and hemorrhage. These were seen in midzonal and central areas. In many central areas, however, nests of liver cells were seen with or without some small fat droplets, the cytoplasm of which was sometimes hyalinized and in a few cases showed hyaline bodies. No intranuclear inclusion bodies were seen.

Some of the bile canaliculi were filled with greenish brown bile. There was moderate proliferation of bile ducts, also definite increase in the periportal fibrous tissue. The involved areas were infiltrated by polymorphonuclear leukocytes, lymphocytes, plasma cells and monocytes.

Pancreas: The cystically dilated duct was lined by a single layer of low columnar epithelium. At the periphery of the dilated duct, some atrophy of the pancreatic acini was seen. Little if any dilation of the smaller ducts or of the main pancreatic duct was found. The acinic epithelium had pale, more or less vacuolated cytoplasm, suggesting fatty or hydropic degeneration. The islets of Langerhans were essentially normal. No inflammatory cell infiltration, fatty replacement or fibrosis of the pancreas was present. The peripancreatic and intralobar fat, however, showed considerable inflammatory involvement. The cytoplasmic outline was lost, and the fat cells were invaded by phagocytes, lymphocytes and plasma cells, which arranged themselves inside the cell membrane. Other groups of similar inflammatory cells formed clumps in the necrotic fat cells. The inflammation was closely related to small veins and arteries.

Kidneys: The glomeruli were quite cellular. The increased cellularity was due to endothelial proliferation and some increase of leukocytic infiltration. The afferent and efferent arterioles were sometimes fairly prominent, owing to proliferation of endothelial cells. The tubules showed cloudy swelling and some vacuolation. A few small areas of scarring were seen, in which the tubules were atrophic and contained hyaline casts. The scarred areas were infiltrated by lymphocytes, plasma cells and monocytes.

In the peripelvic adipose tissue several areas of necrosis infiltrated by lymphocytes, monocytes and foamy macrophages were observed.

Adrenal Glands: The cortical cells showed little if any vacuolation. The cytoplasm was pale pink staining. The medulla was normal. There was considerable congestion assuming a hemorrhagic character. In several areas the periadrenal fat showed early involvement, with a few fat cells here and there being infiltrated by lymphocytes and monocytes. No areas of necrosis were present.

Intestines: Sections of the intestines and the mesentery showed several areas of necrosis of fat infiltrated by macrophages, lymphocytes and plasma cells. Occasionally a lymphocyte was seen phagocytosed in a large macrophage.

Heart: Sections of the heart showed similar lesions in the epicardium.

Spleen: The lymphoid tissue was definitely decreased. The malpighian corpuscles were small and indistinct. There was considerable reticulo-endo-

thelial hyperplasia. A moderate amount of golden brown pigment was seen both inside and outside endothelial cells. While a few stem cells were encountered here and there, no appreciable degree of infiltration with immature cells and no hemopoiesis was present. No Dorothy Reed cells or megakaryocytes could be found. The lymph nodes presented similar reticuloendothelial hyperplasia.

Bone Marrow: There was hyperplasia of the marrow with definite retardation of maturity of the granulocytic series.

Postmortem Bacteriologic Study.—Blood culture gave a heavy growth of *Bacillus coli*.

Final Diagnoses.—Nonsuppurative panniculitis involving the skin, the epicardium and the peripancreatic, periadrenal, perirenal and mesenteric adipose tissue; portal cirrhosis with fatty metamorphosis of the liver; dilatation of the duct of Santorini; hyperplasia of the reticuloendothelial system and the bone marrow; bronchopneumonia; bilateral hydrothorax; terminal acute parotitis; postoperative fibrosis and scarring of the thyroid gland.

COMMENT

This 39 year old Filipino soldier had recurrent fever of seven months' duration, associated with leukopenia and, in the last two months of his illness, with cutaneous lesions that recurred and regressed, leaving no scars.

His history revealed that he had undergone thyroidectomy for adenoma three years previously. Little thyroid gland was left. There was no history of the use of alcohol, tobacco or other drugs.

Prior to his illness he had been given quinacrine hydrochloride for two years, and during his illness he received quinine, sulfadiazine, penicillin and antimony without any apparent change in the course or the outcome of his disease.

The cutaneous lesions observed in this case presented the histologic picture described in other cases reported in the literature and leave no doubt as to the correctness of the diagnosis of nonsuppurative panniculitis.

Miller and Kritzler ^{2a} were the first to report an autopsy of Weber-Christian disease. In their case the lesions of adipose tissue were limited to subcutaneous tissue. Additional findings were: accumulation of fat in, and focal necrosis of, the liver; hydropic degeneration of the adrenal cortex; phagocytosis of red cells in fixed members of the reticuloendothelial system; fat embolism of the lungs.

Spain and Foley ^{2b} were the second to report an autopsy. The patient had chronic glomerulonephritis with uremia. Nonsuppurative lesions of the adipose tissue were found in the skin, the mesentery, the omentum and the pretracheal regions. Foci of fat necrosis were seen in the pancreas, but these did not resemble the subcutaneous lesions. No areas of necrosis were found in the spleen or the liver, but there were fatty changes in the latter.

A case has been reported by Friedman ^{2c} and grouped with these cases by him. The patient had staphylococcic septicopyemia without any visceral lesions of the adipose tissue. The outstanding picture in the panniculus was extensive suppuration caused by staphylococci, and it was quite dissimilar to the microscopic and bacteriologic picture

seen in any of the reported cases of Weber-Christian disease. Therefore, the case may not be considered as representing this syndrome.⁴

Our case showed the most widespread involvement of adipose tissue so far reported in the literature. Nonsuppurative lesions of the fat cells were present in the subcutaneous, epicardial, peripancreatic, periadrenal, perirenal and mesenteric tissue. Hyperplasia of the reticulo-endothelial system and of the bone marrow with definite retardation of the granulocytic series was seen. There were foci of necrosis in the liver with portal cirrhosis and extensive fatty metamorphosis of the liver. The duct of Santorini was dilated.

Our patient did not have uremia or any clinical evidence of renal disturbance. There was no fat embolism of the lungs.

The lesions were those of the early stages of the disease, and no scarring was seen.

The genesis of the disease remains obscure. Quinacrine hydrochloride, quinine, antimony, penicillin and sulfadiazine had been given to our patient sometime during the preceding few months. There was a definite element of dietary deficiency. Yet many patients have been seen who have had most of these drugs and been malnourished without panniculitis developing.

It is interesting to note that a tentative diagnosis of Hodgkin's disease had been made early in the course of the disease but could not be verified by histologic examination of tissue. He was also suspected of having leprosy at one time, but nasal smears, as well as biopsy specimens of the lesions, revealed no evidence of this disease. Similarly, no other organisms or parasites could be found in the lesions.

Disturbances of the metabolism of fat present an intriguing explanation, which would be entirely speculative and hypothetical.

SUMMARY

A case of fatal relapsing febrile nonsuppurative panniculitis (Weber-Christian disease) has been presented. This case showed the most widespread involvement of adipose tissue so far reported in the literature. The lesions were in the early stages and involved the subcutaneous, epicardial, peripancreatic, periadrenal, perirenal and mesenteric fat. There was no suppuration, and no etiologic agents could be determined.

4. Since this article was submitted for publication we have seen a report by H. Ungar (*J. Path. & Bact.* **58**:175, 1946) of a case in which there were widespread lesions of the panniculus showing suppuration and lipogranuloma formation. The patient had chronic streptococcal infection and died of hemolytic streptococcus peritonitis. The outstanding characteristic of the lesions was suppuration due to streptococci. We do not believe that the case should be included under the heading "Nonsuppurative Nodular Panniculitis."

CONGENITAL HYPERTROPHY OF THE NECK OF THE URINARY BLADDER
WITH BILATERAL HYDROURETER, HYDRONEPHROSIS AND
POLYCYSTIC KIDNEY

ADELE S. VAIL, M.D., and TIMOTHY P. STONE, M.D., FRAMINGHAM, MASS.

CONGENITAL obstruction of the urinary tract is now recognized to be a more frequent cause of hydronephrosis than was thought in the past. In a series of 4,903 autopsies of infants and children, Bugbee and Wollstein¹ noted this condition in 0.9 per cent. The obstructive lesion may be found in either the prevesical or the post-vesical tract. Aberrant vessels, strictures of the ureters, ureteral valves, kinks and unusual ureteral insertions are the most frequent causes of prevesical obstruction. Of the causes of postvesical obstruction, it appears that most fall within the category of the urethral valvelike folds, so well described by Young and associates² and by Lowsley and Kirwin,³ which are most frequently found in the region of the verumontanum (colliculus seminalis) and in the posterior part of the urethra. Langebeck⁴ is accredited with the first description of the anomaly. Other reports soon followed (Velpeau⁵); it was, however, many years later that a complete study by Tolmatschew⁶ had much to do with arousing general interest in the subject. Half a century had hardly passed when Young, Fronz and Baldwin² were able to collect 23 similar cases from the literature, to which they added 12 of their own. Six additional cases were reported by Hinman and Kurzmann⁷ and 3 by Kretschmer.⁸ A search of the literature by Lowsley and Kirwin³ brought the total number of cases reported up to 1934 to 133, of which 3 were their own. The presence of velamentous valves in the posterior part of the urethra is perhaps the most frequent but not the only cause of postvesical urinary obstruction. Phimosis, stricture of the meatus of the urethra, hypertrophy of the verumontanum and contracture or hypertrophy of the vesical neck have been mentioned occasionally in connection with congenital hydroureter and hydronephrosis. Hypertrophy of the vesical neck has been the subject of an excellent study by LeRoy,⁹ who recognized that the condition was due to one or another

From the Department of Pathology, Framingham Union Hospital.

1. Bugbee, H. G., and Wollstein, M.: *J. A. M. A.* **83**:1867, 1924.
2. Young, H. H.; Fronz, W. A., and Baldwin, J. C.: *J. Urol.* **3**:289, 1919.
3. Lowsley, O. S., and Kirwin, T. J.: *J. Urol.* **31**:497, 1934.
4. Langebeck: *Mémoire sur la lithotomie*, Thesis, Paris, 1802.
5. Velpeau, A.: *Nouveaux éléments de médecine opératoire*, Paris, J. B. Baillière, 1832, p. 907.
6. Tolmatschew, N., cited by Lowsley and Kirwin.³
7. Hinman, F., and Kurzmann, A. A.: *J. Urol.* **14**:71, 1925.
8. Kretschmer, H. L., and Pierson, L. E.: *Am. J. Dis. Child.* **38**:804, 1929.
9. Le Roy: *Arch. ital. di urol.* **11**:175, 1934.

of the following main causes: hyperplasia and hypertrophy of the peri-urethral glands; hyperplasia of the fibrous connective tissue stroma, most apparent in the submucosa and the muscular coats; hyperplasia and hypertrophy of the muscular fibers; diffuse hyperplasia of all elements of the vesical neck. It is with the latter condition that we are concerned in the case here presented, unusual for the concomitancy of bilateral hydroureter, hydronephrosis and polycystic kidney.

REPORT OF A CASE

A 26 year old secundipara entered the Framingham Union Hospital one month before term after an uneventful pregnancy. Her past history was not relative, and her first child was living and well. In due time labor began and was terminated in a breech extraction. At birth the baby weighed 4 pounds 10 ounces (2,098 Gm.) and measured 12 inches (30.5 cm.) in length. He was cyanotic, and his respirations were "gasping." He was placed in an oxygen bassinet but, despite all attempts at resuscitation, died within one hour and fifty minutes.



Fig. 1.—Urinary bladder, ureters and kidneys, dissected in one block. Note thickening of the neck of the urinary bladder, bilateral hydroureter, hydronephrosis and polycystic kidneys.

Autopsy (Dr. C. G. Tedeschi).—The body was that of a premature baby boy, 12 inches in length and 2,050 Gm. in weight. No evidence of malformation, injuries or lesions of any type was noted with the exception of general cyanosis.

The lungs were atelectatic. About 2 cc. of transparent amber-colored fluid was present in the pericardial sac, but no cardiac abnormalities were observed. The urinary bladder was greatly distended, reaching the level of the umbilicus. The ureters followed a rather tortuous course and were markedly sausage-shaped, owing to alternating dilated and narrowed tracts. The kidneys were greatly enlarged and cystic. The other abdominal organs showed no significant changes.

The kidneys, ureters and urinary bladder were removed together (fig. 1). The right kidney measured 5.5 by 4 by 3.5 cm. and weighed 18 Gm. The left kidney measured 6 by 4 by 3.6 cm. and weighed 19 Gm. The usual structure was completely obliterated by fluctuant saclike formations of different sizes, some of which were empty, while others contained lemon-colored clear fluid. The

calices and pelves were extremely dilated and contained fluid like that in the saccular formations. Each kidney was reduced to a thin layer of tissue from 0.1 to 0.2 cm. in thickness. Tiny cysts were embedded in it. The right ureter measured 7 cm. in length and the left 8.5 cm., and each when opened had a width ranging from 1.0 to 1.5 cm. Both were free from any obstruction. They were lined by smooth gray-pink mucosa.

The urinary bladder measured 6 by 4 by 3 cm. and except for the unusually large size was not remarkable on external examination. It contained about 15 cc. of lemon-colored transparent fluid, and the mucosa was pale gray and glistening throughout. The region of the trigone was clearly made out, and the ureteral openings were promptly recognized. The bladder wall was thickened throughout,



Fig. 2.—Photomicrograph showing an island of epithelial tubules, in disorderly arrangement, embedded in a fibrous stroma in which engorged blood vessels stand out conspicuously (microscope Zeiss, ocular 5, objective 10).

most strikingly in the region of the neck, where it measured from 3 to 5 mm. in thickness. This resulted in marked narrowing of the lumen of the neck, which hardly admitted the finest probe after considerable pressure, regardless of whether the probing was done from the bladder or from the urethra. The latter could be probed without difficulty, and the only pathologic condition in it was hypospadias of moderate degree. Neither testicle was in the scrotal sac.

Sections from both kidneys showed complete obliteration of the usual structure. The most striking pattern was that of islands of epithelial tubules embedded in a fibrous stroma simulating under low power the structure of glandular acini (fig. 2). Some of the tubules were small, and some were dilated into cystic spaces. The smallest tubules were lined by one or more layers of low cuboidal epithelial cells. The cystic spaces were also lined with epithelial cells, which,

however, showed flattening and less frequent stratification. Glomeruli could hardly be recognized; the few present showed striking thickening of the basal membrane of the loops, resulting in a compact appearance (fig. 3). The stroma in which the tubules were embedded was dense and poor in cells in some areas, while in other areas it was loose and edematous, composed of an interlacing of connective tissue fibers, histiocytes and fibroblasts. Engorged capillaries, either isolated or in clumps, were present in the latter areas, and small extravasations of red blood cells were noted. Bundles of smooth muscular fibers were present here and there, pointing, as is generally thought, to immaturity of renal tissue. The calices and pelves were not unusual except for some thickening of the epithelial lining in some places and thinning in other places, with marked vascular conges-



Fig. 3.—Photomicrograph of an area of the same field as that in figure 2, at higher magnification, showing the presence of occasional glomeruli and tubules dilated to cystic dimensions (microscope Zeiss, ocular 10, objective 4).

tion and patchy accumulations of lymphocytes which at times had an arrangement suggesting the structure of lymph follicles.

Changes of the same type were present in the ureters, which in addition showed, deep in the mucosa, small islands of epithelial cells (cell nests of Brunn), some of which were excavated at the center. The thickening of the neck of the urinary bladder appeared in the microscopic sections to be due to a diffuse hyperplasia of all the elements composing the neck. The characteristic disposition in layers and the intimate structure of the muscular fibers were well preserved, but the myofibrils were obviously more numerous and thicker than usual, with scattered evidence of nuclear giantism. Between the bundles of muscular fibers the fibrous stroma was homogeneously thickened, but in no areas was scarring or active inflammatory reaction suggested.

The number of openings of the prostatic tubules into the urethra did not appear to be more numerous than one expects in a premature newborn baby. In sections at different levels, from 34 to 51 prostatic tubules were counted, a number considered to be within normal limits by Lowsley.¹⁰ From the histologic study it was concluded that the cause of the urinary obstruction was diffuse hypertrophy and hyperplasia of all elements of the urinary neck.

COMMENT

The outstanding features of the case can be summarized as follows: bilateral hydroureter and hydronephrosis, polycystic kidneys and thickening of the neck of the urinary bladder, leading to a practically complete urinary obstruction, in a newborn boy. The urinary obstruction in this case falls into the category of "diffuse hyperplasia of all elements of the vesical neck" of LeRoy's classification. An interesting feature of the case is the polycystic kidneys. Giordano¹¹ published a similar case in which a newborn baby presented ectopy of the heart, extrophy of the urinary bladder, deviation of the spine and left talipes equinovarus. Failure of the involution of the first few generations of the glomeruli and tubules and loss of their connections with the connecting tubules constitute the most favorably accepted explanation of polycystic kidney. Obliteration of the usual renal structure with replacement by adenomatous structures and muscular fibers in the stroma of the parenchyma is evidence in our case also of a deep developmental renal defect.

SUMMARY

A case of systemic malformation of the entire genitourinary tract, with cryptorchidism, hypospadias, hypertrophy of the neck of the urinary bladder, bilateral hydroureter and hydronephrosis, and polycystic kidneys is reported.

10. Lowsley, O. S.: *Am. J. Anat.* **13**:293, 1912.

11. Giordano, A.: *Arch. ital. di urol.* **11**:158, 1934.

Obituaries

NEWTON EVANS, M.D.

1874—1945

On Dec. 19, 1945, at Los Angeles, Dr. Newton Evans died of carcinoma of the stomach. Thus ended the career of an eminent pathologist, a pioneer in this field as well as an active exponent of scientific medicine in the West.

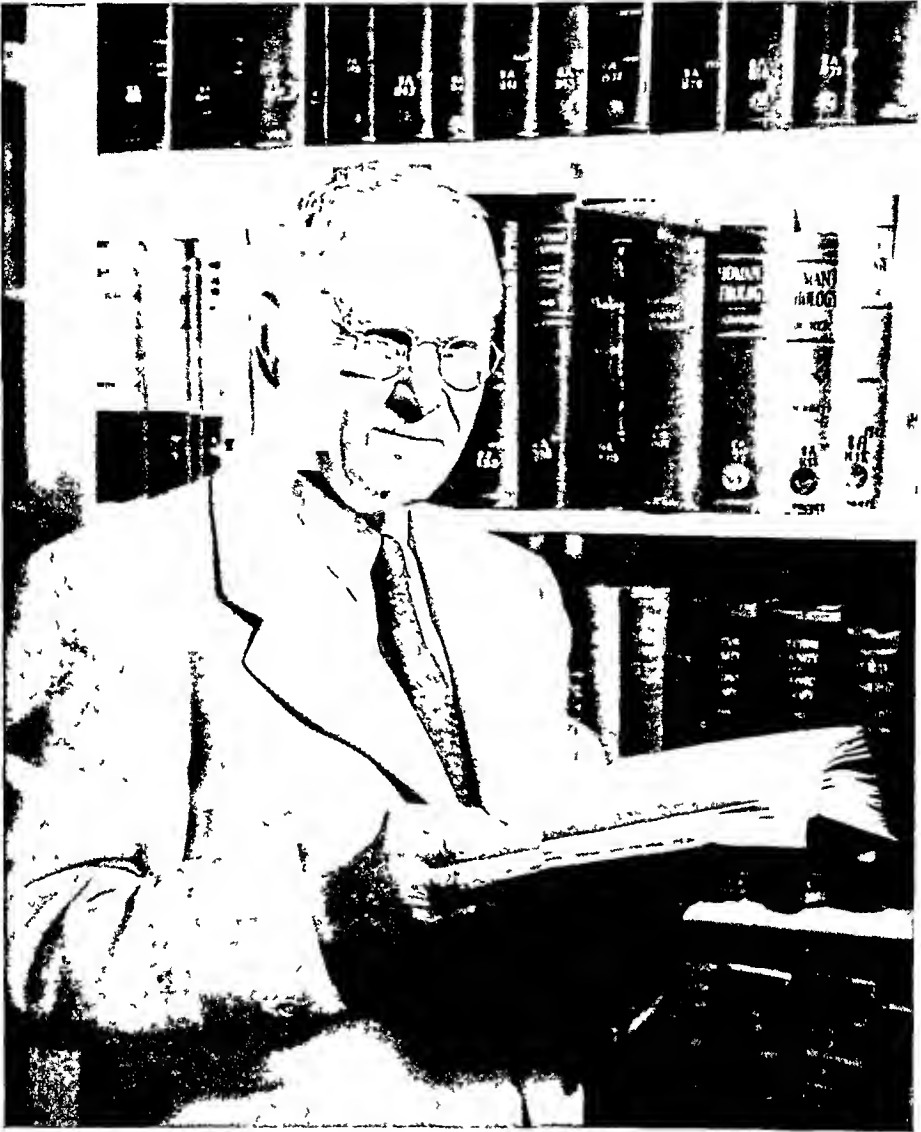
Dr. Evans was born in Hamilton, Mo., June 1, 1874, the son of William and Emma (Newton) Evans. The family moved to Nebraska, where he spent his boyhood and adolescence. He attended Union College at Lincoln, where he received his A.B. degree in 1895. In 1896 he enrolled in the American Medical Missionary College at Battle Creek, Mich., but spent his senior year at Cornell University School of Medicine, where he was graduated in 1900.

He returned to Battle Creek and began his professional career as an instructor in histology and pathology. Here he was joined in marriage to Cora Mildred Deming. Three children were born to this union, one of whom met an accidental death in adolescence; the two living are Dr. William Dustin Evans and Emma Elizabeth (Mrs. Howard A. Ball). The pressure of mundane obligations forced him to enter private practice in Kentucky and Tennessee for a time, but from 1908 to 1911 he served as professor of pathology and head of the department in the University of Tennessee, where he acquired a reputation as a stimulating and inspiring teacher.

In 1914 Dr. Evans answered an emergency call to a then small and struggling medical school in California, agreeing to teach one course of pathology. When the semester was concluded, he returned to his practice. But his success as a teacher was such that the students unanimously insisted on his return, a call which he found himself unable to refuse. The following year he was appointed professor of pathology and head of that department of the College of Medical Evangelists, a position which he held for about thirty years, his tenure terminating only with his death. During this interval he sponsored and actively promoted the cooperative plan in medical education.

In 1928 he was appointed chief of the department of pathology of the Los Angeles County Hospital. His vision of the place of the laboratory in the field of scientific medicine led to the rapid development of this service, until it became the fountain head of the scientific spirit of the institution as well as the source of help in the solution of the

many diagnostic and therapeutic problems arising in this large hospital. During this interval he was active in many medical and scientific organizations, both local and national. His published contributions



Newton Evans

NEWTON EVANS, M.D

1874-1945

were concerned largely with the rarer granulomatous diseases (torulosis, blastomycosis and coccidioidal granuloma), the pathologic aspects of diseases of the cardiovascular system, the liver and the kidney, and

particularly cancer. His personal interests were thus clearly reflected, for it was his conviction that in the degenerative diseases and in cancer progress must be made if current medicine was to achieve any important progress.

He retired from this position at the Los Angeles County Hospital a short year and a half before his death. He had looked forward to spending his last years as counselor to the administration of the medical school to which he had given much of his time and effort. His death came as a profound loss to the school, one which is still acutely appreciated at a time of rapid and important changes.

Dr. Evans stands out preeminently as a teacher. He was not dramatic or sensational. At times students were inclined to loll at their microscopes—but usually not for long. Questions for idle minds were soon forthcoming. And woe to the student whose lenses were murky and mirror dusty! A lesson not soon forgotten was taught by the professor himself, who took the student's place at the microscope, making the optics shine with his own clean handkerchief.

As a teacher he took great interest in the library of the school, becoming its outstanding scientific patron, for he believed that both doctors and students should seek the answers to their questions in contemporary literature.

It was his steadfast, unfailing purpose to awaken interest, to inspire lagging spirits and to encourage the feeble efforts of young men in the scientific way. It may truthfully be said that his own achievements might have been much greater had he not been more interested in seeing others get ahead. The work he was happy to do; the honors he was willing others should have.

Dr. Evans maintained his humble, teachable attitude to the end. Not always easily convinced, he became a forceful supporter of a project when its objectives were made clear and its worth appreciated.

If one watchword would characterize his daily life, it would be "Aequanimitas." His unruffled spirit, his unfailing fidelity and transparent honesty were his way of life. It is this portrait of him that will long live in the memory of his devoted pupils.

CYRIL B. COURVILLE, M.D.

Notes and News

Appointments, Etc.—C. F. Geschickter has been named professor of pathology and parasitology and director of the department in Georgetown University School of Medicine, Washington, D. C.

Everett L. Bishop has been promoted from associate professor to professor of pathology (neoplastic diseases) at Emory University School of Medicine, Atlanta, Ga.

Charles F. Branch, formerly professor of pathology and dean at Boston University School of Medicine, has joined the staff of the American College of Surgeons as assistant director. Dr. Branch will be particularly concerned with the work of the cancer committee of the college, which includes surveys and approval of cancer clinics and the establishment of cancer detection centers.

Robert A. Moore, professor of pathology at Washington University School of Medicine, St. Louis, has been appointed dean of the school.

J. T. Syverton, associate professor of bacteriology in the University of Rochester, has been appointed professor and head of the newly formed department of microbiology in the Louisiana State University School of Medicine, New Orleans.

Deaths.—Oscar T. Schultz, at one time professor of pathology and bacteriology at the University of Nebraska and director of the Nelson Morris Institute at Michael Reese Hospital, Chicago, and recently pathologist of St. Francis Hospital, Evanston, Ill., died on March 7, 1947, at the age of 70.

George H. Weaver, formerly professor of pathology in Rush Medical College and physician at the Durand Hospital of the John McCormick Institute for Infectious Diseases, Chicago, died on April 19 in his eighty-first year.

Society News.—The American Public Health Association announces that its seventy-fifth annual meeting will take place in Atlantic City, Oct. 6 to 10, inclusive, 1947. Exhibits and the scientific program will point up progress in public health over a seventy-five year span.

The next meeting of the American Society of Clinical Pathologists will be held in Chicago, October 27 to 30 next, with headquarters at the Drake Hotel. October 28 and 29 will be devoted to scientific sessions and October 30 to the annual seminar.

The Alabama Association of Pathologists met in Birmingham on Monday, April 12, 1947. The guest speaker was Sidney Madden, professor of pathology, Emory University School of Medicine. The clinical pathology conference was opened by Reginald Fitz, of Harvard Medical School, Boston. Arthur Purdy Stout, professor of surgical pathology, Columbia University, College of Physicians and Surgeons, was moderator of the tumor seminar.

International congresses are scheduled for next July as follows: Cytology, Stockholm, Sweden, July 10 to 16; Microbiology, Copenhagen, Denmark, July 20 to 26; Physiology, Oxford, England, July 21 to 25.

American Society for the Study of Arteriosclerosis.—Plans have been completed to organize the society. Clinicians and experimentalists interested in a concerted intensified investigation of all phases of arteriosclerosis are invited to join the organization and are urged to communicate as soon as possible with

Dr. O. J. Pollak, Wilmington General Hospital, Chestnut and Broom Streets, Wilmington 14, Del. The constituting meeting has been called for Monday, June 9, 1947, at 9 a. m. at the Hotel Strand, Atlantic City, N. J. The members of the organizing committee are: G. R. Herrmann, Galveston, Texas; W. B. Kountz, St. Louis; R. A. Katz, New Orleans; J. B. Wolfe, Philadelphia; L. C. Duff, Montreal, Canada; H. Goldblatt, Los Angeles; W. C. Hueper, New York; I. H. Page, Cleveland.

Change of Date for Examination by the American Board of Pathology.

The Board will hold an examination in Chicago on October 24 and 25 next. Applications will not be accepted after September 15. Inquiries should be directed to Dr. Robert A. Moore, secretary-treasurer, American Board of Pathology, Washington University School of Medicine, St. Louis 10.

Honorary Members of New York Pathological Society.—At the last meeting of the New York Pathological Society, Pierre Masson, Peyton Rous and Ludvig Hektoen were elected honorary members in recognition of their contributions to the science of pathology, and Francis Carter Wood for contribution to pathology and service to the Society.

Course on Nuclear Physics.—The University of California Medical School, in association with the University Extension, announces a course on nuclear physics as applied to the biologic and medical sciences, to be given from June 30 through July 18, 1947. It will consist of didactic lectures, laboratory demonstrations and seminars for round table discussions, and will be open to persons interested in medical and biologic research. For information write to Dr. Stacy R. Mettier, University of California Medical Center, San Francisco 22.

Books Received

LA CULTURA IN VITRO DEL MIDOLLO OSSEO: PROBLEMI DI FISIOPATOLOGIA EMATOLOGICA STUDIATI CON LA TECNICA DELLA CULTURA DEI TESSUTI. By Aminta Fieschi and Giovanni Astaldi. Prefazione di Cesare Frugoni. Pp. 309, with 132 illustrations (10 in color). Price 1,000 lira. Pavia, Italy: Tipografia del Libro, 1946.

This book, the result of extensive work carried out through ten years, does not simply summarize numerous publications. It completes the researches with new material and a thorough discussion of the special and general questions arising in the course of the investigations. After the literature is extensively reviewed, a simplified method of culture is described. The material comprised specimens of bone marrow in normal condition and specimens obtained in cases of pernicious anemia, chronic myeloid leukemia, chronic lymphatic leukemia, acute leukemia and familial erythroblastic anemia (Cooley's anemia). Of the numerous points of interest, encountered at every step of the studies, only a few may be mentioned here: the duration of the phases through which the normoblast matures from the basophilic erythroblast to the final erythrocyte; the plasma cells of type Unna-Marschalko, which originate not from lymphocytes but from a special plasmoblast; the direct histioblastic origin of the megaloblast, which is gradually replaced by forms transitional to normoblasts after the onset of liver treatment; leukemic histogenesis, which is extensively discussed. An essential difference between chronic and acute leukemia was found in the short survival of the cells of the former and the long survival, one month or more, of the latter. The cells characterizing the single cases of acute leukemia and the surviving explant may best be classified as hemocytoblasts, monocytoid cells and paramyeloblasts. These cells, though surviving, show no developmental faculties and may be termed leukemic cells, whereas the cells of chronic leukemia have preserved the capacity of differentiation and maturation. No cell deserving the designation of leukemic cell can be demonstrated with certainty in the chronic forms. The fundamental question of the nature of leukemic growth, whether simply hyperplastic or neoplastic, viewed from the cultural experiments, is discussed in a clear way, without any tendencies toward precocious inferences. Important in this respect is the observation that new lymphoblasts are formed from the reticulum in explants derived from subjects of chronic lymphatic leukemia. Concerning the leukemic cells of acute leukosis the authors, without taking a decisive choice, hint at the possible interpretation as "pre-hemocytoblastic" in the sense of Ferrata, which by the preservation of more of the histioid character could account for the longer survival. This conservative attitude the authors retain for every question and thereby keep the reader always aware of the unsolved problems and the controversial points. This advantage makes the book a most valuable guide in research and most instructive reading for any one interested in the more intricate parts of normal and morbid hemopoiesis. The numerous illustrations and the beautiful colored plates are a most important help in understanding the contents.

DIE INTERSTITIELLE NEPHRITIS. By Privatdozent Dr. M. H. U. Zollinger, professor at the pathologic institute of the University of Zurich; with a foreword by Prof. Dr. H. von Meyenburg. Pp. 264, with 87 illustrations and 3 tables. Price, 28 Swiss francs. New York: S. Karger, 1945.

To former generations of pathologists and clinicians "interstitial nephritis" was a familiar term. But after Volhard and Fahr emphasized the morphologic changes in the parenchyma of the kidneys, the concept of a primary type of interstitial nephritis fell into disrepute. The author of this monograph of 264 pages

has attempted, with much zeal, to raise this type of change to a place of importance in the pathology of the kidneys. He attempts to separate cases of interstitial nephritis into groups on an etiologic basis in order to discover the types of reaction of the interstitial tissue of kidneys which have undergone different forms of injury. He believes that the interstitial changes are often the most significant factor in prognosis. In sharply limiting his investigations to a single histologic fraction of the kidneys, he has been led to neglect the functionally more important changes in the parenchyma, the fact that the kidneys function by the coordinated activity of all their parts and that most diseases of these organs are accompanied by clinically and pathologically important extrarenal pathologic processes. In the preparation of this monograph he studied the lesions in kidneys from more than 200 patients and the changes induced experimentally in a large number of rabbits.

This volume is divided into 14 chapters. The first chapter is a brief introduction to the subject. In the second chapter the author discusses the interstitial changes occurring in the kidneys in scarlet fever, infectious jaundice, diphtheria, typhoid, measles and other acute infectious diseases, tuberculosis, syphilis and "lymphogranuloma." To this is added a section on cryptogenic infections in which the renal interstitial changes gave the only clue to the nature of the disease. Chapter 3 is concerned with interstitial nephritis in disturbances of protein metabolism. The acute forms of this type include the renal changes that occur with hemolysis, with specific protein disintegration, with hypochloremia, toxemia of pregnancy and exogenous poisons. The chronic forms occur in plasmocytic multiple myeloma, lipoid nephrosis, amyloidosis, gout, renal rickets and in conditions of uncertain etiology. The author found intertubular edema especially characteristic of this group and believed it to be due to the presence of abnormal protein in the tissues. Chapter 4 deals with the interstitial renal changes that accompany urinary stasis and ascending nonpurulent nephritis. Chapter 5 is concerned with neoplastic, including leukemic, infiltrations of the kidneys. In chapter 6 the author discusses interstitial *Begleitnephritis*, i. e., the interstitial changes that accompany focal and diffuse glomerulonephritis, etc. Chapter 7 is devoted to spontaneous and experimental interstitial nephritis of animals. In the remaining chapters, there are discussed the nomenclature and classification of interstitial renal processes; the normal and the pathologic histology of the interstitial tissue of the kidneys; the pathogenesis of interstitial nephritis; the relation between clinical and anatomic findings, and the therapy of the anuria in interstitial nephritis.

The seven and one-half closely printed pages of bibliography will be useful to any one interested in the pathology of the kidneys. The 87 illustrations lack the sharpness and distinctness to which American readers are accustomed. At the end of the book is a large folded table in which are given the details of the histopathology of the stroma of the kidneys in about 96 cases classified under 11 headings.

This monograph represents a prodigious amount of labor in reviewing literature and studying a large amount of pathologic material. It will have little interest for clinicians. Pathologists will regret that so much labor has been expended on such a limited phase of renal pathology, although this phase has been presented with remarkable completeness.

GYNECOLOGICAL AND OBSTETRICAL PATHOLOGY WITH CLINICAL AND ENDOCRINE RELATIONS. By Emil Novak, M.D., D.Sc. (Hon. Dublin), associate in gynecology, Johns Hopkins Medical School; gynecologist, Bon Secours and St. Agnes Hospitals, Baltimore. Second edition. Pp. 570, with 542 illustrations (15 in color). Price \$7.50. Philadelphia and London: W. B. Saunders Company, 1947.

Shortages of labor and paper delayed publication of the second edition of this valuable book. The text has been brought well up to date. More than 100 new illustrations, microscopic and gross, have been added. Many of the chapters are profusely illustrated, numerous variations in pattern being depicted. The book is warmly recommended to all students of gynecologic and obstetric pathology. It should be available in all pathologic laboratories. It maintains well the high standards of pelvic pathology set in the Johns Hopkins Medical School.

LINDAU-VON HIPPEL DISEASE WITH HEMANGIOBLASTOMA OF THE SPINAL CORD AND SYRINGOMYELIA

THOMAS D. KINNEY, M.D.

AND

PATRICK J. FITZGERALD, M.D.

BOSTON

IN 1926 LINDAU¹ drew attention to the frequency with which angioblastic tumors of the retinas and single or multiple angioblastoma of the central nervous system were associated with visceral defects. Hemangioblastoma of the spinal cord was present in 4 of the 15 cases originally reported by Lindau, and since that time 7 similar cases have been described. It is the purpose of this communication to report the twelfth and thirteenth cases of hemangioblastoma of the spinal cord accompanying Lindau-von Hippel disease. In these cases there was shown, in addition, syringomyelia, and they are the eleventh and twelfth cases to present this combination of lesions.

Fuchs² (1882) was the first to describe angiomatosis of the retinas, and Collins³ (1894) was the first to describe the retinal lesions microscopically. Von Hippel⁴ (1895) placed the emphasis on the ophthalmologic signs and symptoms, and since then angiomatosis retinae has been associated with his name. Seidel⁵ was the first to note the association of cerebellar and retinal angioblastomas although it was not until after Lindau's article in 1926 that the association became widely known. Cushing and Bailey⁶ gave the tumors further prominence and distinguished them histologically from various other vascular tumors.

All types of vascular neoplasms of the spinal cord are uncommon. In a series of 557 intraspinal tumors reported by Rasmussen, Kernohan and Adson,⁷ only 64 intramedullary neoplasms were described and but 5 were of vascular origin.

From the Mallory Institute of Pathology, Boston City Hospital.

1. Lindau, A.: *Acta path. et microbiol. Scandinav.*, 1926, suppl. 1.

2. Fuchs, E.: *Arch. f. Augenh.* **11**:440, 1882.

3. Collins, E. T.: *Tr. Ophth. Soc. U. Kingdom* **14**:141, 1894.

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Wolf and Wilens⁸ in a review of the literature found only 11 authentic cases of hemangioblastoma of the spinal cord, and added 1 case. They stated that in 5 cases, including their own, there was associated Lindau's disease. In their case syringomyelia and syringobulbia were also shown, and they expressed the belief that it was the fourth case reported in which cystic spinal cord changes occurred with hemangioblastoma and associated Lindau-von Hippel disease.

Hemangioblastoma of the spinal cord occurs frequently in association with Lindau-von Hippel disease, also as an isolated tumor. As has been noted, Lindau¹ found the combination to be present in 4 of 15 cases. Additional cases of hemangioblastoma of the spinal cord associated with Lindau-von Hippel disease have been described.⁹ As far as can be determined, the total number of cases of hemangioblastoma of the cord described in the literature (exclusive of the present cases) is 22, in 11 of which it was associated with Lindau-von Hippel disease, and in 10 of these 11 cases syringomyelia was also present. Isolated hemangioblastoma of the cord with syringomyelia has been described in at least 4 cases: Pinner,¹⁰ Tannenberg¹¹ (case II), Russell,¹² Turner and Kernohan.¹³

REPORT OF CASES

Dr. John Conlon supplied the clinical data in this case.

CASE 1.—A 49 year old white man stated that the onset of his illness occurred in 1919 when, at the age of 29, he noticed, for the first time, that the vision of his left eye was impaired. This condition progressed steadily until by 1935 there was complete loss of vision in the eye. The patient experienced no other symptoms until December 1934, when he began to suffer from sharp jabbing pains in his head on lateral motion. Within a short time he became dizzy when walking, and within a period of two months the dizziness became so pronounced that it was necessary for him to remain in bed. He stated that objects did not revolve but that it was impossible for him to walk straight or to stand. During this period the patient began to suffer from severe occipital and frontal headaches. There was occasional vomiting, unaccompanied by nausea. He was admitted to a hospital, where he was found to have angiomatosis of the right and the left eye. This

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9. (a) Davison, C.; Brock, S., and Dyke, C. G.: *Bull. Neurol. Inst. New York* **5**:72, 1936. (b) König, E., and Schoen, H.: *Beitr. z. klin. Chir.* **170**:239, 1939. (c) Wolff, K., and Donat, R.: *Ztschr. f. urol. Chir. u. Gynäk.* **43**:272, 1937. (d) Harbitz, F.: *Acta path. et microbiol. Scandinav.* **11**:442, 1934. (e) Knodel, G.: *Virchows Arch. f. path. Anat.* **281**:886, 1931. (f) Craig, W. M.; Wagener, H. P., and Kernohan, J. W.: *Arch. Neurol. & Psychiat.* **46**:210, 1941. (g) Schuback, A.: *Ztschr. f. d. ges. Neurol. u. Psychiat.* **110**:359, 1927.

10. Pinner, A. W.: *Arb. a. d. Geb. d. path. Anat. Inst. zu Tübingen* **9**:118, 1914.

11. Tannenberg, J.: *Ztschr. f. d. ges. Neurol. u. Psychiat.* **92**:119, 1924.

12. Russell, D. S.: *J. Path. & Bact.* **35**:103, 1932.

13. Turner, O. A., and Kernohan, J. W.: *Arch. Neurol. & Psychiat.* **46**:444, 1941.

was most marked in the left eye, which was completely blind. The angioma in the right eye was most prominent in the upper nasal quarter of the retina. There was lateral nystagmus, and the reflexes were hyperactive, especially in the left side. There was past pointing to the right with the left hand, as well as clumsiness of the left hand. The patient stood on a wide base, Romberg's sign was positive, and the gait was staggering both to the right and to the left side. A roentgenogram of the skull at this time was essentially normal. A diagnosis of Lindau's disease was made.

On March 20, 1935, cerebellar exploration was performed. The surgeon described the right cerebellar hemisphere as being normal in appearance. In the upper portion of the left cerebellum a cystic area 3 cm. in diameter was found. Associated with this area was a tumor mass which was adherent to the dura in the vicinity of the torcular of the occipital bone. The cyst was opened and all the tumor mass was removed except for a small portion which was adherent to the dura and the torcular. The pathologic diagnosis of the excised tumor was "hemangioma." The postoperative course was uneventful, and the patient was discharged, symptom free, on April 14.

Following the operation, the base of the brain was given 8 exposures to roentgen rays. With the exception of occasional headaches, the patient remained symptom free for four years; then the dizziness suddenly returned together with moderately severe generalized headaches. There was no nausea, vomiting or change in visual efficiency. It was felt that additional surgical intervention was useless, and the patient was admitted to a veteran's hospital for nursing care. The physical examination made at the hospital showed little beyond a weak, emaciated man who had a slightly hyperactive left knee jerk, blindness of the left eye, angioma of the right eye and varicocele of the left side. The patient's condition gradually deteriorated, and he died on July 14, 1940.

During the patient's various hospitalizations, the following significant laboratory data were recorded: The urine was acid, with a specific gravity of 1.022, no albumin and no sugar. Microscopic examination of the urine gave negative results early during his hospitalization and later showed 7 to 8 red blood cells per high power field, no white blood cells and no casts. The Hinton test of the blood was negative. The blood nonprotein nitrogen was 44.4 mg. per hundred cubic centimeters.

Autopsy.—The body was that of a normally developed but poorly nourished white man. The right pupil measured 0.3 cm. and the left 0.2 cm. The left pupil was gray and opaque. There was a posterior median cervical scar 3.5 cm. long and 2.5 cm. wide, also a scar posterior to the left ear 3.5 cm. in length. The heart was normal. There were areas of consolidation throughout both lungs. The pancreas weighed 370 Gm. and measured 29 cm. in length, 9 cm. in width and 4 to 6 cm. in thickness. The organ appeared to be made up almost entirely of cysts measuring from 0.1 to 3 cm. in diameter, with occasional areas of normal-appearing pancreatic tissue between the cysts. The walls of the cysts were paper thin, gray, translucent, and the cysts contained clear straw-colored fluid. The left kidney weighed 490 Gm. and the right 180 Gm. The capsular surface of the left kidney was distorted along the middle and lower portions by a large nodular mass of red and yellow tissue which measured 10 by 5 by 6 cm. When the kidney was sectioned, it was found that the mass had replaced the entire lower two thirds of the kidney and that it extended into and distorted the pelvis of the kidney. The mass itself was made up of golden yellow soft tissue and criss-crossed by streaks of firm white fibrous tissue. Throughout the mass were areas of softening and hemorrhage. At the upper pole of the same kidney was a smaller

and discrete, but otherwise similar, mass which measured 1.0 cm. in diameter. The remainder of the renal parenchyma showed the usual renal structure. Throughout the right kidney were several similar tumor nodules, which varied from 0.5 to 1.5 cm. in diameter. The left pampiniform plexus was markedly dilated. The remainder of the organs other than the brain showed no gross lesions.

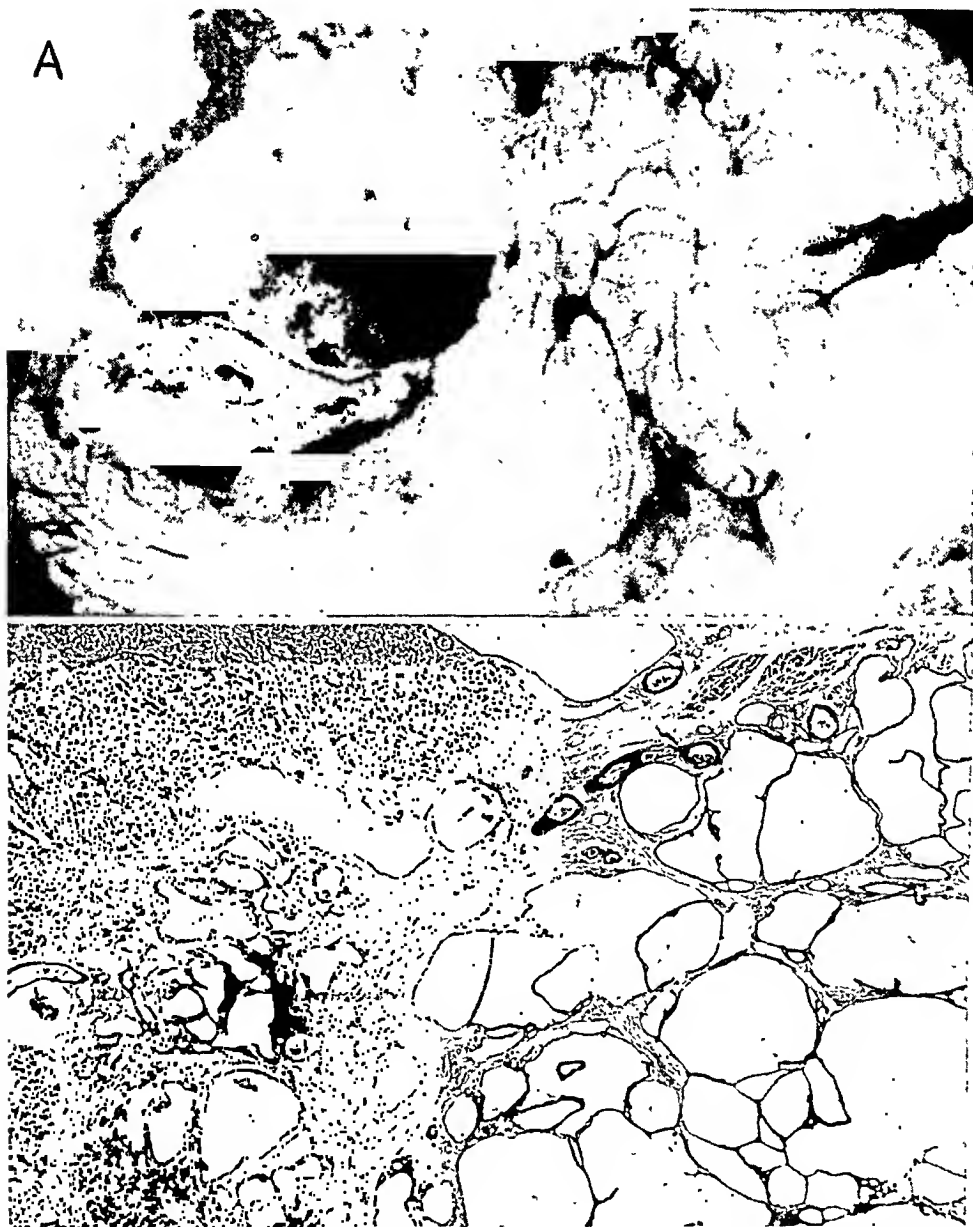


Fig. 1 (case 1).—*A*, left hemisphere of the cerebellum, showing a tumor with cystic spaces containing blood. *B*, section of the cerebellar tumor showing the variation in size of the cysts and some highly cellular areas. Phosphotungstic acid-hematoxylin stain; $\times 15$.

The brain weighed 1,575 Gm. There was moderate flattening of the convolutions of the cerebrum, and the sulci were narrowed. Coronal sections of the

cerebrum did not reveal any abnormalities. There was a dark red cystic mass in the posterior part of the left cerebellar hemisphere, measuring 5.5 by 5.0 by 3 cm. It was made up for the most part of irregular cavities, 1 to 2.5 cm. in diameter. These cavities were smooth-walled and filled with blood. The remainder of the mass was made up of similar but smaller cavities measuring 0.1 to 0.5 cm. (fig. 1 *A*).

The spinal cord showed no gross changes except at the level of about the third to fourth cervical segments. Here one posterior horn was more conspicuous and larger than usual, and the adjacent gray matter was gray-purple. No distinct cavity was seen.

Microscopic Examination.—In the lungs near the larger bronchi were a few small cysts, the walls of which were made up of fibrous tissue covered by a layer of low cuboidal epithelium. There was moderate congestion of the alveolar capillaries, and there were focal areas in which the alveolar spaces were filled by polymorphonuclear leukocytes. The pancreas contained innumerable cysts, varying in size, the walls of which were composed of fibrous tissue lined by low columnar epithelium. In the areas in which these cysts were most numerous, there was considerable fibrosis, with partial replacement of pancreatic acini and compression of neighboring acini. In the regions in which cyst formation was less marked, the usual pancreatic structure was present. The islets of Langerhans did not appear to be involved.

Sections from the tumor masses in the kidneys presented similar histologic pictures. The tumor cells were large and round, with abundant clear cytoplasm and small dark basophilic nuclei. The blood vessels were large and numerous. Considerable hemorrhage and necrosis occurred throughout the tumor. The neoplasm was invasive, and there was no evidence of encapsulation. Sections from the uninvolved renal parenchyma showed no changes.

The retina of the left eye was completely destroyed; the usual layers could not be identified, as they were replaced by an almost continuous band of bone in which there was a small amount of marrow with a few stem cells and nucleated red cells. There were two relatively large tumor masses of thin-walled vessels, and in the intervacular tissue were mononuclear cells containing fat and brown pigment granules. In the choroid there was an increase in the number of chromatophore cells. There was atrophy of the left optic nerve with loss of myelin, extensive gliosis and an increase of connective tissue in the surrounding meninges and perivascular tissue. There appeared to be a decrease in the nerve cells of the retina of the right eye. No tumor was present in the section obtained from the right eye.

The cerebellar tumor was made up of many thin-walled blood vessels lined by endothelial cells. These blood vessels varied in size from capillaries to cavernous vessels, grossly visible (fig. 1 *B*), and red blood cells were present in some lumens (fig. 2 *A*). There were many mononuclear phagocytic cells containing lipid substance in the intravascular tissue. Many poorly formed minute vascular channels, devoid of blood, were lined by endothelial cells. In some places the cells of the intervacular tissue were abundant, mononuclear and possessed of considerable cytoplasm (fig. 2 *A*). There were many large cavities, the walls of which were lined by endothelial cells and which contained no erythrocytes. Reticulum stains showed the capillaries and large vessels to be well outlined and the intervacular tissue to contain much reticulum (fig. 2 *B*). No normal cerebellar tissue was found in the tumor mass.

In the cord, at the level of the third to fourth cervical segments there was an irregularly shaped cavity extending from the posterior commissure into the dorsal

horn laterally. No definite tumor cells were seen in relation to this cavity. In the wall of the cavity were many macrophages, and there was some increase of astrocytes in adjacent tissue. In the cord, at the level of the fourth to fifth

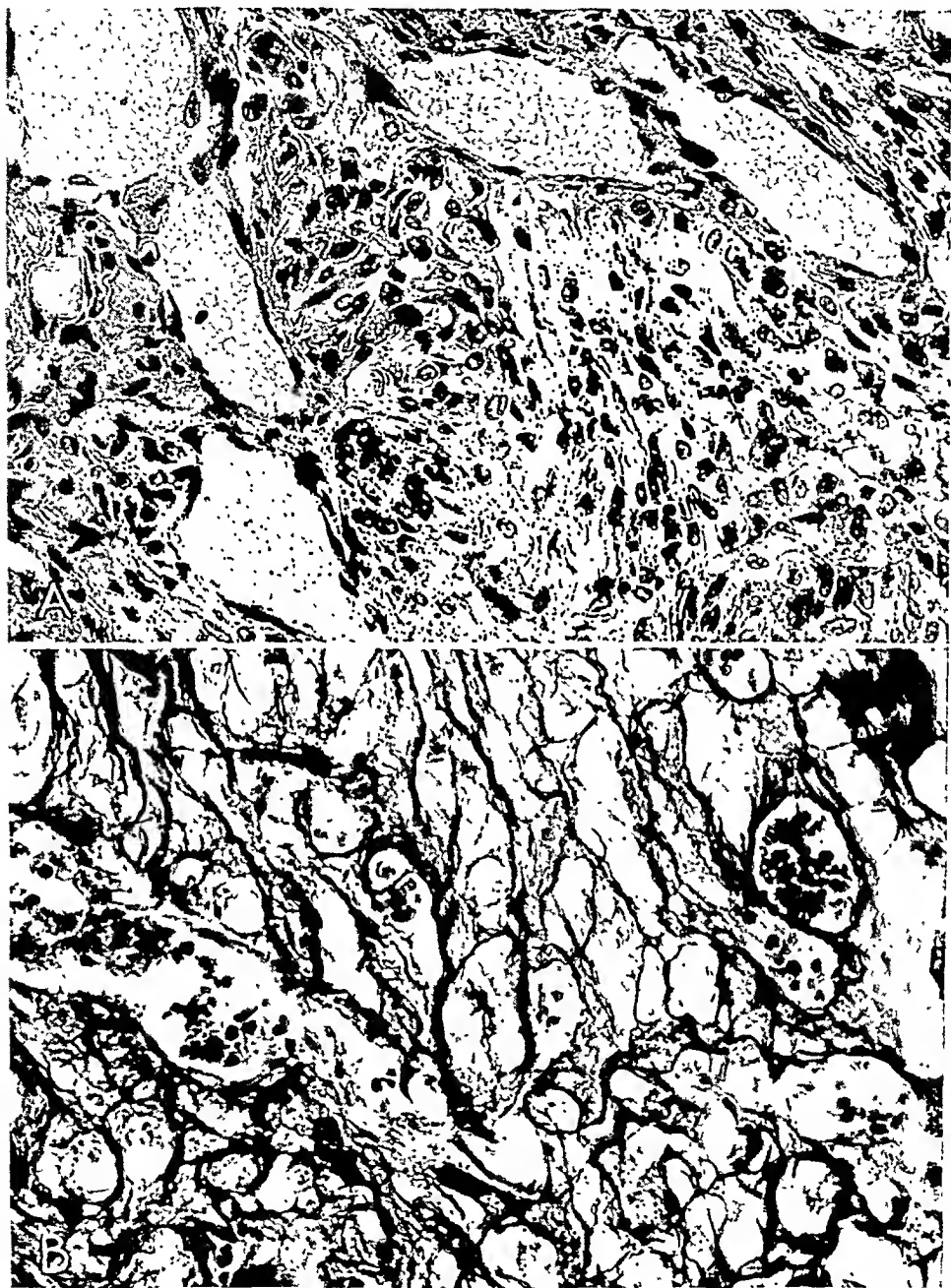


Fig. 2 (case 1).—*A* shows the blood-filled spaces of the cerebellar tumor and the mononuclear cells in the intervacular areas. Phosphotungstic acid-hematoxylin stain; $\times 400$. *B* shows the abundance of reticulum in the vessel walls and in the intervacular tissue of the cerebellar tumor. Perdrau silver stain; $\times 500$.

cervical segments there was tumor in the meninges, in the columns of Clarke and Burdach and in the dorsolateral portion of the cord extending from the roots' entry to within 2 to 3 mm. of the midline and occupying almost all of the afore-

mentioned columns. Adjacent to the tumor were cavities showing no lining cells but surrounded by large astrocytes. The tumor showed the usual vascular channels lined by endothelium with abundant lipid in the cytoplasm. There was an abundance of mononuclear cells together with a few fibroblasts in the intervascular tissue.

Anatomic Diagnoses.—Hemangioblastoma of the cerebellum, left hemisphere, and the cervical portion of the cord; syringomyelia of the cervical portion of the cord; hemangioblastoma of the left eye, with destruction of the retina and bone formation; cysts of the pancreas; cysts of the lungs; bilateral hypernephroma; bronchopneumonia of the lower lobes of the right and left lungs, and varicocele of the left side.

In case 1 the patient experienced impairment of vision at the age of 29 years, and it was not until sixteen years later that signs of cerebellar tumor appeared. One of the striking features of this case is the completeness of the Lindau-von Hippel complex, as the cerebellum, the retinas, the spinal cord, the lungs, the pancreas and the kidneys were involved. This is one of the few cases recorded in which the visceral lesions produced clinical signs, since the microscopic hematuria and the varicocele of the left side were probably related to the hypernephroma. The lesion of the spinal cord is of considerable interest because of the association of hemangioblastoma of the spinal cord and syringomyelia with the classic Lindau-von Hippel lesions of the cerebellum and the retina. There were no neurologic signs or symptoms which could be attributed to the involvement of the spinal cord.

CASE 2.—M. Z., a 27 year old single white woman, was admitted to the Boston City Hospital, Dec. 7, 1936, because she was unable to walk without assistance. The past history is interesting in that at the age of 2 years she underwent surgical immobilization of the upper thoracic region for presumable Pott's disease. When she was 25 years of age, radical mastoidectomy was performed on the left side at the Boston City Hospital for chronic discharge of the ear, and a polyp was removed from the posterior wall of the external meatus. The patient stated at that time that she occasionally suffered from dizziness but that she never fell. She also complained of partial blindness of the left eye, and she had vision in only the lower visual field. Examination of the left fundus showed old retinitis proliferans with detached retina. The right eye was normal. The history of her family was not remarkable.

The patient dated the onset of her present illness to June 1933, three and a half years before the final admission; at that time she began to suffer from hiccup, which usually came on at night and which was occasionally accompanied with nausea and vomiting. She stated that she had always been deaf in the left ear. Shortly after the mastoidectomy, in 1935, she began to suffer from headaches. Two months before admission, in 1936, she had daily headaches which were almost continuous. One year before admission she became completely blind in the left eye. Four weeks before admission she noticed weakening of both legs and numbness of the toes. This appeared to be more marked on the right than on the left side.

On admission her temperature was 98.4 F., the pulse rate 92 and the respiratory rate 22 per minute, and the blood pressure 90 mm. of mercury systolic and 70 diastolic. The patient was a poorly nourished and poorly developed woman in

no great discomfort. There was marked sharp flexion deformity in the region of the first and second dorsal vertebrae, and there was an old healed operative scar over this deformity. The spinal column was otherwise not remarkable. There was a discharge of the left ear, and a mastoidectomy scar was present on the left side. The left eye was blind, and there was complete separation of the retina with evidence of old retinal hemorrhage. The right optic disk showed choking of 4 diopters, with small hemorrhages visible about the optic disk. There was a large beaded vessel, apparently an artery, extending laterally and inferiorly from the disk. This vessel disappeared at the margin of a circular white area located at about 8 o'clock, which was slightly larger than the optic disk and was surrounded by a broken narrow rim of black pigment. Two large vessels were seen at the lateral side of the area, but their course could not be followed farther. The cranial nerves were otherwise not abnormal. The upper extremities were not remarkable, but there was marked weakness of the lower extremities, more marked on the right. There were poorly sustained left ankle clonus and bilateral ataxia. The Kernig signs were not present. There was no definite level of sensory loss, but there seemed to be anesthesia to pain and touch in about the distribution of the fifth lumbar nerve bilaterally, and there was markedly diminished vibration sense at both ankles and absence of position sense in the legs. Both biceps reflexes and the left radial reflex were hyperactive. The abdominal reflex of the lower left quadrant was absent. The right knee jerk was diminished and the left increased. The left ankle jerk was more active than the right. There were positive bilateral Babinski responses. A diagnosis of Lindau's disease was made, and a cerebellar exploration was carried out. At operation a cystic tumor was found in the medulla and another in the cord. It was thought to be unwise to attempt removal of either of these tumors. Following operation, the patient's temperature was 102 F.; the pulse rate was 120 to 180. She complained of headache, later became unresponsive, and died two days after operation.

On admission the hemoglobin was 80 per cent (Sahli); the red blood cell count was 4,100,000 and the white blood cell count 6,000 per cubic millimeter. The examination of the urine gave negative results. The Hinton test of the blood was negative. Lumbar punctures on three occasions showed initial pressure between 90 and 100 mm., with no rise on jugular compression, but a rise of 200 mm. on abdominal compression. A cisternal puncture showed an initial pressure of 350 mm., with a rise to 500 mm. and a rise to 450 mm. on jugular and abdominal compression, respectively.

The specimens of cerebrospinal fluid obtained from lumbar punctures and from the ventricular puncture made at the time of operation were clear, slightly yellow and without clot. They showed no white blood cells, but on some occasions a few red blood cells. Fluid obtained by lumbar puncture gave a 4 plus reaction in the Pandy test and contained between 420 and 600 mg. of protein per hundred cubic centimeters. The Lange colloidal gold curve was 1222333432, and the Wassermann test was negative. Fluid obtained by cisternal puncture gave a 1 plus reaction in the Pandy test and contained 150 mg. of protein per hundred cubic centimeters.

Roentgenograms of the mastoid processes showed sclerosis and destruction of bone on the left side. A roentgenogram of the skull showed no abnormality. A roentgen examination of the chest revealed normal lung fields, enlarged hilar glands and marked distortion of the spinal column.

Postmortem Examination.—The body was that of a fairly well nourished white woman showing generally immature development. There was a recent

operative scar in the occipital region extending 21 cm. from side to side and 8 cm. caudal from the midpoint of the lateral incision. There was marked kyphosis of the upper dorsal portion of the spinal column. With the exception of the central nervous system, there were no gross lesions demonstrable in any of the organs of the body. There was an oval operative bony defect 8 by 5 cm. in the occipital region. The exposed sutured dura was covered by a thin seropurulent exudate. The brain weighed 1,370 Gm. There was considerable flattening of the convolutions with narrowing of the sulci. The meninges at the base of the brain and over the posterior portions of the temporal lobes were covered by thin yellow purulent exudate. This exudate was also present above the tentorium at the junction of the sagittal and transverse fissures. When frontal sections were cut, bilateral hydrocephalus was demonstrated, and each of the lateral ventricles measured 2.7 by 2 cm. Beneath the ependyma of both the lateral and third ventricles were multiple petechial hemorrhages averaging 0.1 to 0.2 cm. in diameter. The cerebellum itself was normal, but the posterior portion of the fourth ventricle, the foramen of Magendie and the posterior portion of the cisterna magna were involved by a neoplastic mass measuring 1.7 by 1.5 by 1 cm. This mass apparently arose from the floor of the fourth ventricle and there was only slight involvement of the cerebellum. It was firmer in consistence than the surrounding brain tissue, and its color was pink-gray. Two small cystic channels appeared ventrally near the attachment of the tumor to the floor of the fourth ventricle; one measured 0.4 by 0.3 cm. and the other 0.4 by 0.8 cm. The pituitary gland was covered by thick purulent fluid.

The arches of the first and second cervical vertebrae had been removed. There was ankylosis of the first six dorsal vertebrae with marked kyphosis, and the peak of angulation was at the level of the fifth dorsal vertebra. The cord correspondingly showed marked angulation at this level, and it was narrowed here so that it measured 1 by 0.65 cm. in cross section.

The entire cervical portion of the cord was swollen, and this was most marked in the lower five segments. The upper four segments were fluctuant. Multiple sections of the cord showed the tumor of the fourth ventricle extending caudad through the left nucleus of Burdach and the left substantia gelatinosa Rolandi into the left posterior horn and left posterior column of the cervical and dorsal segments of the spinal cord. The tumor extended downward to the level of the eleventh dorsal segment. It contained a continuous cystic cavity extending from the first cervical to the sixth dorsal segment and a small cyst, 0.1 cm. in diameter, in the left posterior column at the eleventh dorsal segment. In addition, there was a small and apparently independent tumor nodule, measuring 0.2 cm. in diameter, in the left lateral column at the level of the fifth cervical segment.

The posterior third of each orbit was removed. On the left the choroïd was detached with the exception of a small area laterally. The remainder lay in the posterior chamber and was adherent to the posterior surface of the lens. In the right eye there was a small pink elevation, 2 cm. in diameter, at the extreme right of the choroid.

Microscopic Examination.—In the medulla there was a tumor nodule arising from the posterior medullary velum and protruding into the fourth ventricle. The nodule was composed of many capillaries, as well as larger vessels of varying size lined with endothelium and containing red blood cells. Between the vessels was a fairly heavy stroma varying from isolated fibroblasts to many mononuclear cells, some containing lipid. The tumor in the cerebellum was similar to that seen in case 1. The nodule extended into the lateral portion of the fourth ventricle but it

was impossible to determine from the material available whether the area postrema was involved. Ventral and lateral to the tumor nodule was a large cavity, the walls of which were formed by proliferated astrocytes. Along one margin of the cavity were a large focus of polymorphonuclear and mononuclear cells and a few

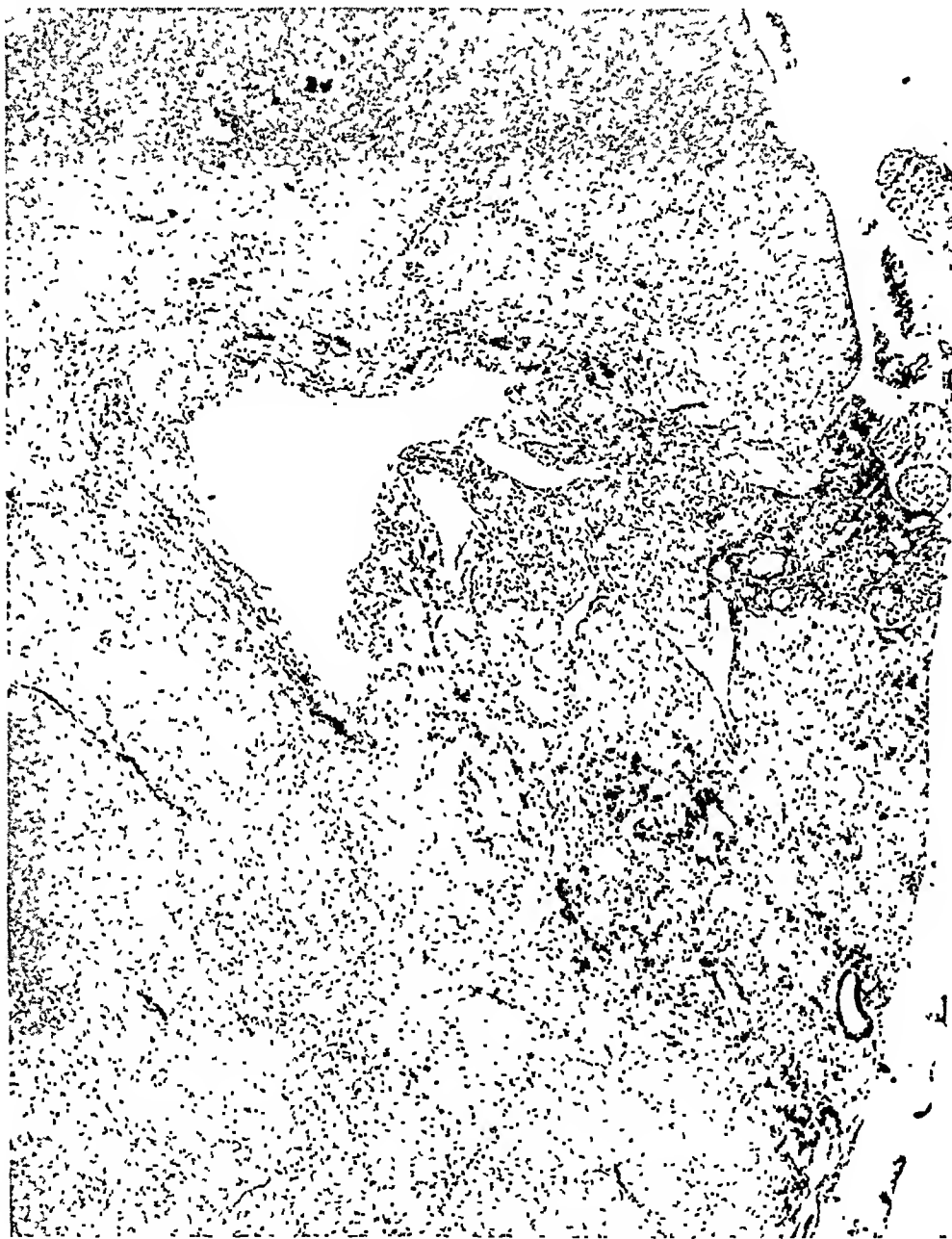


Fig. 3 (case 2).—Spinal cord at the level of the eighth cervical segment, showing tumor and a syrinx. Hematoxylin and eosin stain; $\times 15$.

adjacent foci of capillaries filled with red blood cells which were surrounded by a zone of histiocytes. The cavity occupied the dorsal lateral portion of the medulla and extended into the restiform body on one side. The ependyma around the

fourth ventricle showed several petechial hemorrhages, and there was fibrino-purulent exudate in the substance of the medulla, particularly around the larger vessels, and the ependyma.



Fig. 4 (case 2).—Spinal cord at the level of the first dorsal segment, showing a syrinx with a wall of glial tissue and absence of tumor. Hematoxylin and eosin stain; $\times 15$.

A small nodule of tumor tissue similar to that described in the foregoing paragraph was found in the stalk of the pituitary gland.

Histologic sections revealed tumor in the left posterior horn and the left half of the posterior column from the first cervical to the eleventh dorsal segment (fig. 2B) and a small, apparently independent nodule, measuring 0.2 by 0.175 cm., in the left lateral column at the fifth cervical segment. The tumor tissue of the spinal cord was identical to that of the floor of the fourth ventricle and impinged on a cystic cavity which extended from the first cervical to the sixth dorsal segment (fig. 3). There were small areas of cavitation in the neighborhood of the tumor in the left posterior horn at the fourth and fifth cervical segments, in both anterior horns at the seventh cervical segment, in both posterior horns at the eighth cervical segment, in both anterior horns and the right posterior horn at the first and third dorsal segments. The walls of the cavity showed hyperplasia of large glial astrocytes, and no tumor cells were present (fig. 4). Areas of necrosis were seen in the gray matter of the left side from the eighth to the tenth dorsal segments and in both anterior horns at the eleventh dorsal segment, and there was marked gliosis in the posterior columns from the eighth to the eleventh dorsal segment, corresponding to the angulation of the gibbus of the spinal column.

There was polymorphonuclear infiltration of the meninges of the cord extending from the medulla oblongata downward to the second lumbar segment. This infiltration was massive down to the level of the fifth dorsal segment and only slight from the fifth dorsal to the second lumbar segment. The choroid plexus was covered with purulent exudate.

Permission was obtained to remove only the posterior portion of each retina. The retina of the left eye showed detachment with folding, as well as hyperplasia of large chromatophores and prominent capillaries, but there was no definite tumor formation. The retina of the right eye showed no tumor and no detachment. It is possible that the vascular and capillary hyperplasia seen in the left retina represented tissue taken from the edge of a tumor similar to that of the medulla oblongata and the cord.

Several small cysts were present in the lungs, and the majority of these were located in small clusters at the periphery of the lung. The walls of the cysts were made up of fibrous tissue and were lined by low columnar epithelium. There were foci of alveoli filled with polymorphonuclear cells, bacteria and debris.

There were a few small cystic spaces in the pancreas, which were lined by low columnar epithelium showing clear cytoplasm and large nuclei with diffusely scattered chromatin. The cysts appeared to cause no distortion of the usual pancreatic structure.

Sections taken from the vertebral column at the point of acute angulation showed destruction of bone and replacement by fibrous tissue. There was no evidence of tumor or inflammation.

Anatomic Diagnoses.—Hemangioblastoma involving the floor of the fourth ventricle and the spinal cord (first cervical to eleventh dorsal segment); syringomyelia (first cervical to sixth dorsal segment and at the eleventh dorsal segment); syringobulbia; cysts of the pancreas; cysts of the lungs; purulent meningitis; marked kyphosis with ankylosis of the first to the sixth dorsal vertebrae; healed fibrous pleuritis of the upper and middle lobes of the right lung; bronchopneumonia of the lower lobe of the left lung.

The clinical history in case 2 is similar to that in case 1 in that the first symptom was impairment of vision followed later by signs and symptoms referable to a disturbance of the central nervous system.

The marked kyphosis was a confusing factor in this case. It was first thought that there was compression of the cord from Pott's disease and that there were associated tuberculomas of the cerebellum and the eye. However, the diagnosis of Lindau's disease was made and subsequently confirmed by cerebellar exploration.

As far as can be determined, this is the twelfth case to be reported in which syringomyelia was associated with hemangioblastoma of the spinal cord and the thirteenth case in which hemangioblastoma of the cord was associated with the Lindau-von Hippel syndrome. Syringobulbia was also present.

COMMENT

Lindau-von Hippel disease is uncommon. Symptoms usually appear between the second and fourth decades, and the disease shows no particular sex incidence. Lindau¹⁴ stated that 25 per cent of the patients suffering from von Hippel disease will show symptoms due to associated cerebellar lesions. He pointed out that a familial factor can be demonstrated in approximately 20 per cent of all cases of this syndrome. Thomsen,¹⁵ from his studies, expressed the belief that the disease is transmitted by a single dominant factor, not sex linked. Möller¹⁶ was able to demonstrate 6 single or combined retinal and cerebellar tumors in three generations, and on the basis of the pronounced hereditary factor he was able to diagnose correctly, for the first time postoperatively, an intracranial hemangioblastoma. With a family history of the disease or the demonstration of retinal angioma and with the presence of signs of disturbance of the cerebellum or of the fourth ventricle, a probable diagnosis of Lindau-von Hippel disease can be made.

Numerous investigators have postulated a congenital origin for hemangioblastoma. The work of Sabin¹⁷ and Karlefors¹⁸ has shown that in the third fetal month a vascular mesenchyme is situated in or adjacent to the posterior end of the fourth ventricle (velum medullare posterior), and Lindau expressed the belief that since the cerebellar hemispheres develop at about this period a part of the original vascular mesenchyme might be drawn into the cerebellar hemispheres. Vascularization of the retina, ingrowth of the mesodermal elements and the branching anlage of the pancreas also take place in the third fetal

14. Lindau, A.; Sargent, P., and Collins, E. T.: *Proc. Roy. Soc. Med.* **24**:363, 1931.

15. Thomsen, O., cited by Möller.¹⁶

16. Möller, H. V.: *Acta ophth.* **7**:244, 1929.

17. Sabin, F. R.: *Anat. Rec.* **13**:199, 1917.

18. Karlefors, J.: *Die Hirnhäuträume des Kleinhirns*, Stockholm, Norstedt & Söner, 1924.

month, and it is possible that the other associated developmental defects occasionally seen in patients with Lindau-von Hippel disease occur at this period.

Cushing and Bailey⁶ divided vascular tumors of the central nervous system into two groups: angiomas and angioblastomas, a subdivision which has been generally recognized. The angiomas were considered to be vascular malformations consisting of fully formed blood vessels with an intervening stroma of normal nerve tissue. Angioblastomas were considered to be true tumors capable of slow progressive growth, which rarely show mitoses or giant cell formation and which do not metastasize or spread along the ventricular or subarachnoid spaces. These tumors are composed of an extensive new growth of vascular channels containing red blood cells and lined with endothelial cells. Reticulum stains show a characteristic pattern of outlined capillaries and intercapillary bridges. Considerable variation is seen in the amount of intervascular tissue; some tumors show capillary arrangement with little or no intervening tissue, while others reveal marked intervascular cellularity with many minute, poorly formed vascular spaces, usually empty, and lined by either well formed adult endothelial cells or by more primitive cells—the latter being presumably immature endothelial cells. The tumor cells typically have an oval vesicular nucleus and pale cytoplasm containing a few vacuoles. Nucleoli are not uncommon. Mitoses are rare. Occasionally the lipoid content of the interstitial substance is marked and overshadows the vascular picture. This lipoid material is contained in large mononuclear phagocytic cells. Lindau expressed the belief that the vascular tumor with its large blood-filled spaces caused pressure necrosis of the nerve tissue with consequent liberation of lipoid, which was subsequently phagocytosed by the endothelial elements of the tumor. The tumors frequently show areas of regressive changes, such as edema, hyalinization, calcification and occasionally fatty degeneration.

A further differentiation of the types of hemangioblastoma was made by Cushing and Bailey⁶ on the basis of structure. The type showing predominance of narrow vascular channels with thin septums was described as capillary; that showing widely dilated channels, as cavernous, and a type showing a relative paucity of vessels and predominance of cellular elements was designated as cellular. This further subdivision of hemangioblastomas does not appear to be valid, as all types of hemangioblastoma are frequently seen in different sections of the same tumor, although one type may predominate.

Cerebellar hemangioblastomas in general comprise less than 1 per cent of intracranial tumors. In patients with Lindau-von Hippel disease their presence usually gives rise to cerebellar symptoms some years

after the ocular changes. The average age at onset of the cerebral lesions is 32 years (Lindau). These tumors may be accompanied by hemorrhage and edema. Lindau collected from the literature and his own material 40 cases of cystic hemangioblastic tumor of the cerebellum, and he gives the impression that in most instances the tumor is cystic although in a few it is solid or only partially cystic. The neoplasm manifests itself by the usual signs and symptoms of a cerebellar tumor, and there is nothing distinctive that allows it to be clinically differentiated from other cerebellar tumors. It has been emphasized by Dandy¹⁹ and Cushing⁶ that in the cystic type the small tumor nodule usually found on the cyst wall must be removed to prevent refilling of the cavity, as aspiration of the cyst is not sufficient for cure. The tumor in case 1 was partially cystic.

Hemangioblastoma may rarely occur in other portions of the rhombencephalon as in case 2. This is the finding in 2 of the previously reported cases of Lindau-von Hippel disease with involvement of the cord (Knodel²⁰ and Schuback²⁸).

When a lesion of the spinal cord is present with Lindau-von Hippel disease, it is usually a hemangioblastoma. It lies in the dorsal half of the cord, usually in the posterior columns and adjacent to the posterior septum. The tumor has occurred at all levels of the cord but shows a predilection for the cervical and lumbar regions. It has been observed in the cauda equina and in nerve roots. The fact that the Lindau-von Hippel complex is present in approximately half of the reported cases of hemangioblastoma of the cord suggests the possibility that closer examination of the spinal cord in cases of Lindau-von Hippel disease would reveal an even higher incidence of hemangioblastoma of the cord.

The syringomyelic cavities associated with hemangioblastoma of the spinal cord are surrounded by a dense glial wall, and they are not lined by ependymal cells. Davison, Brock and Dyke²¹ expressed the belief that the cavities are caused by the tumor's destroying or softening of the cord or by its pressing on the intraspinal arteries, with circulatory interference and subsequent cyst formation. Bielschowsky and Unger²⁰ expressed the belief that the cavity and concomitant vascular lesion were congenital anomalies. Lindau considered the origin of the syringomyelia to be similar to that of the formation of a cerebellar cyst: i. e., transudation of fluid from the vessels of the tumor with formation of cysts and compression of adjacent glial tissue resulting in a wall of compressed glia without an ependyma. There was marked cavity formation throughout the cord and medulla in case 2, and to a lesser

19. Dandy, W. E.: *The Brain*, in Lewis, D.: *Practice of Surgery*, Hagerstown, Md., W. F. Prior Company, Inc., 1944, vol. 12, sect. 12, p. 640.

20. Bielschowsky, M., and Unger, E.: *J. f. Psychol. u. Neurol.* **25**:173, 1920.

degree in the cord in case 1. In both cases the walls of the cavities were composed of dense glial tissue, and there was no evidence of an ependymal lining. It is of interest to note that distinct areas of typical syringomyelia were found in which there were no associated vascular lesions.

The ocular lesion is a hemangioblastoma of the retina similar to the vascular tumors found elsewhere. Symptoms from the retinal tumor generally appear at about the age of 25 years, although occasionally the onset is in childhood, but rarely does it occur after the age of 45 years.²¹ It is stated that retinal angiomas are twice as frequent in males as in females.⁸ At first, usually only slight impairment of vision of one eye is noted. Later the other eye may show involvement, or it may remain free from the disease. The ophthalmoscopic picture (Craig, Wagener and Kernohan^{9c}) in the early stage is that of a round red angiomatous mass in the periphery of the retina, and issuing from the mass are two large dilated vessels, artery and vein, running parallel to each other and to the optic disk. Occasionally diffuse dilatation of all retinal vessels without the angiomatous mass is present.

The retinal lesion is progressive, though its rate of growth is variable, and the vision gradually lessens as the retinal and subretinal edema from the tumor gives rise to local and generalized detachment of the retina, hemorrhage, gliosis, secondary glaucoma and blindness. The detachment of the retina and the reactive gliosis and connective tissue proliferation lead to a puzzling secondary ophthalmologic picture that obscures the primary process and is most commonly mistaken for Coats's "exudative retinitis" (Lindau). Calcification and rarely ossification may supervene and leave a monumental stigma visible on roentgen examination of the orbit (Davison, Brock and Dyke^{9a}). In case 1 there were hemangioblastoma, calcification, and ossification with myelopoiesis of the left retina; in case 2 there was detachment of the left retina, and although the diagnosis was made clinically, no tumor was demonstrated conclusively at autopsy, as only a portion of each eye could be removed.

The accompanying visceral lesions are rarely vascular in origin, and most frequently they take the form of cysts of the pancreas and the kidney. The kidney, and rarely the adrenal gland, may show "hypernephromata." In 6 of Lindau's¹ original 15 cases there were "hypernephroid" tumors of the kidneys and others have been described since.²² Cahill, Melicow and Guerry²³ have described a case in which the renal

21. Brandt, R.: *Arch. f. Ophth.* **106**:127, 1921.

22. Wolf and Wilens.⁸ Davison and others.^{9a} König and Schoen.^{9b} Craig and others.^{9c}

23. Cahill, G. F.; Melicow, M. M., and Guerry, Du P., III: *Tr. Am. A. Genito-Urin. Surgeons* (1942) **35**:271, 1943.

tumor was a hemangioblastoma. Less commonly tumors of the epididymis and angioma of the liver are found.

In general, the visceral lesions cause no symptoms, but the patient of Davison, Brock and Dyke^{9a} had abdominal complaints, and at operation a polycystic tumor of the lesser peritoneal sac attached to the pancreas was demonstrated. Microscopic examination revealed a cystadenoma of the pancreas. In case 1 the bilateral hypernephromas were associated with microscopic hematuria and with varicocele of the left side.

SUMMARY

Two cases of Lindau-von Hippel disease with hemangioblastoma of the spinal cord and syringomyelia are presented. The literature is reviewed, and the nature and the genesis of the lesions are discussed.

ROLE OF LYMPHATIC VESSELS IN THE TRANSMISSION OF LIPASE IN DISSEMINATED PANCREATIC FAT NECROSIS

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SO-CALLED pancreatic and peripancreatic fat necrosis is supposedly due to the splitting of neutral fat into glycerol and free fatty acids by pancreatic lipase which has escaped from the injured pancreas. The free fatty acids are thought to combine subsequently with calcium in the tissue and tissue fluids to form insoluble calcium soaps, and it is these products which give rise to the opaque white areas in the fat depots of the abdominal cavity and elsewhere.

Fat necrosis may follow many types of injury of the pancreas, such as acute pancreatitis, primary and metastatic carcinoma of the pancreas, obstruction of the pancreatic ducts and trauma. Indeed, Opie¹ pointed out that fat necrosis bears somewhat the same relationship to pancreatic disease that jaundice does to diseases of the liver.

The mode of transmission of lipase from the pancreas to the sites of fat necrosis has given rise to much speculation, and a review of the literature revealed that the question is still unsettled.

The purpose of the studies reported in this paper was to determine the role of the lymphatic vessels in fat necrosis and to endeavor to demonstrate that these channels are the primary means of transmission of escaped pancreatic lipase.

REVIEW OF THE LITERATURE

Pancreatic and peripancreatic fat necrosis has been mentioned by a number of the early writers, including Schmidt² (1818), Virchow³

From the Mayo Foundation.

Abridgment of a thesis submitted to the Faculty of the Graduate School of the University of Minnesota in partial fulfilment of the requirements for the degree of M. S. in Internal Medicine, in March 1941.

1. Opie, E. L.: *Diseases of the Pancreas*, ed. 2, Philadelphia, J. B. Lippincott Company, 1910, pp. 176-199.

2. Schmidt, cited by Flexner, S.: *Johns Hopkins Hosp. Rep.* 9:743, 1900.

3. Virchow, R., cited by Henke, F., and Lubarsch, O.: *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1929, vol. 5, pt. 2, pp. 358-371.

(1852) and Ponfick⁴ (1871), but Balser⁵ gave his classic gross and microscopic description of what he termed *Fettenekrosen* in 1882. He thought the necrosis occurred because overgrowth of fat tissue cut off the blood supply.

Fitz⁶ in 1889 first pointed out the intimate relationship of fat necrosis to disorders of the pancreas. He described a number of cases of acute pancreatitis associated with fat necrosis.

In 1890 Langerhans⁷ substantiated Fitz's ideas by producing fat necrosis experimentally. He did this by injecting extracts of rabbit pancreas intraperitoneally in dogs. Langerhans also showed by chemical means that fatty acids and calcium were both present in the necrotic patches, probably in combination as calcium soaps.

In 1897 Flexner⁸ showed in an experimental study that the small regions in which fat necrosis had occurred in animals had a much higher content of lipolytic substance than the surrounding fat.

Wells⁹ in 1903 produced experimental fat necrosis in dogs and cats by intraperitoneal injection of extracts of hog pancreas and also of solutions of commercial pancreatin. Both grossly and microscopically these necrotic patches were exactly like those in human beings. Wells found that if his extracts and solutions were heated to about 71 C., all lipolytic activity was lost, and so it was thought that the active substance was an enzyme. Wells pointed out that, although other workers had failed to produce fat necrosis with solutions of trypsin weak in or devoid of lipolytic substance, it could not be definitely stated that lipase alone was responsible for fat necrosis, because at that time, on account of technical difficulties, it was impossible to separate the two enzymes.

For years it was thought that fat necrosis depended on the action of both trypsin and lipase. The trypsin supposedly killed the fat cells first and then the lipase split the neutral fat. Neal and Ellis,¹⁰ however, showed that lipase alone could cause extensive fat necrosis. They proved this by intraperitoneal injection of extracts of hog pancreas which had no tryptic activity and with lipolytic extracts of dried peanuts and sunflower seeds, using dogs.

The exact nature of the material contained in the regions of fat necrosis is controversial. It has been mentioned before that Langerhans concluded from his chemical and histologic observations that free fatty acids and calcium were both present, probably in combination as calcium soaps. It is generally agreed, however, that it is a difficult problem to

4. Ponfick, E., cited by Neal, M. P., and Ellis, M. M.: *South. M. J.* **23**:313, 1930.

5. Balser, W.: *Virchows Arch. f. path. Anat.* **90**:520, 1882.

6. Fitz, R. H.: *M. Rec.* **35**:197, 1889.

7. Langerhans, R.: *Virchows Arch. f. path. Anat.* **122**:252, 1890.

8. Flexner, S.: *J. Exper. Med.* **2**:413, 1897.

9. Wells, H. G.: *J. M. Research* **9**:70, 1903.

10. Neal, M. P., and Ellis, M. M.: *South. M. J.* **23**:313, 1930.

distinguish between the various types of fat and lipid crystals chemically or histologically.

According to Herbert,¹¹ the necrotic patches contain neutral fat, fatty acids and calcium soaps. Her tests showed that relatively small amounts of the fatty acids were in actual combination with calcium in spite of the fact that these patches contain more calcium than the surrounding fat.

Opie produced fat necrosis in cats by ligating both pancreatic ducts. He not only produced necrosis in the usual sites of abdominal fat by this technic but also found evidence of disseminated fat necrosis in the pericardial, subpleural and subcutaneous fat. According to Opie, clinical cases of fat necrosis associated with pancreatic disease in which similar observations were made have been reported in the literature, and he felt that pancreatic lipase was carried to the sites of necrosis in both the abdominal and the thoracic cavity by the lymphatics.

Rostock,¹² a German worker, reviewed the literature on the transmission of lipase in fat necrosis and pointed out that the earlier workers courted three principal hypotheses. They thought that transmission of lipase occurred by (1) direct contact, (2) blood stream transport of either lipase or pancreatic acinous cell emboli and (3) lymphatic transport. Rostock concluded from his studies that most of the evidence favored the hypothesis of transmission by way of the lymphatics.

In order to prove this point more conclusively, he carried out an interesting study in which he used material taken from necrotic regions which had been produced in dogs. When these pieces of tissue were placed in strong hydrogen peroxide, the lymph channels were delineated because oxygen bubbles were liberated as the result of the catalytic action of lymph. Rostock was able to demonstrate microscopically that lymph vessels were present in the tissue adjacent to the sites in which fat necrosis had occurred.

PRESENT STUDY¹³

It occurred to me that if the hypothesis concerning the lymph channels was correct, a close association between the lymphatic vessels and sites of fat necrosis would be shown if a suitable lipolytic material were mixed with a lymphatic-delineating substance and injected intraperitoneally in rats. If, on the other hand, it was not true, no such association would be shown.

Wells and other workers had used solutions of commercial pancreatin to produce fat necrosis in animals, and this seemed the best lipolytic material for my purpose.

11. Herbert, F. K.: *Brit. J. Exper. Path.* **9**:57, 1928.

12. Rostock, P.: *Beitr. z. klin. Chir.* **138**:171, 1926.

13. The work was done in the Section on Experimental Bacteriology of the Mayo Foundation under the direction of Dr. H. E. Robertson.

For the delineation of the lymph channels it was decided to use a suspension of finely particulate graphite called "hydrokollag 300."¹⁴

Preliminary Studies.—Several large normal white rats were killed with ether and necropsy was performed in order to review the normal anatomy of the rat.

In the second group of rats an intraperitoneal injection of a 4 per cent solution of pancreatin¹⁵ in isotonic solution of sodium chloride was given. After twenty-four hours the animals were killed, and examination revealed many small necrotic regions throughout the fat depots of the peritoneal cavity and also in the subpleural and mediastinal fat.

The suspension of finely particulate graphite was injected alone intraperitoneally in other rats to determine whether it had any lipolytic activity. The animals were killed at the end of twenty-four hours, and necropsy was performed. Neither gross nor microscopic examination showed any evidence of fat necrosis. The lymph channels throughout the abdominal cavities and viscera of these rats, however, were delineated as fine black weblike lines. It was also apparent that in the rat there are lymph channels between the peritoneal cavity and the mediastinal lymph nodes similar to those found in the dog by Higgins and Graham.¹⁶ The lymphatics associated with the internal mammary and other mediastinal blood vessels were delineated, and the mediastinal lymph nodes were black with graphite, while the nodes observed in the normal animal were pink.

Major Study.—Twenty-five large white rats were used for this study. An intraperitoneal injection of a mixture of the solution of pancreatin and the graphite suspension was made. Each animal received 6 cc. of the 4 per cent solution of pancreatin in isotonic solution of sodium chloride mixed with 1 cc. of the graphite suspension.

All of the animals survived for at least four hours. Nine of the rats were found dead after eighteen hours, but 16 animals were alive and appeared to be in good condition. Four of the remaining 16 rats were killed eighteen hours, 9 twenty-four hours, 2 twenty-six hours and 1 forty-eight hours after injection.

Gross Examination: The 25 animals were all examined. The peritoneal cavities were opened and found to contain from 3 to 8 cc. of dirty gray fluid. Sites in which evidence of typical fat necrosis was found were numerous and varied in size from pinpoint diameter to 0.5 cm. Evidence of necrosis was found throughout the omental, mesenteric, perirenal and subperitoneal fat. The epididymis of each male rat was also involved. The lymph channels were delineated by the graphite as previously described, and there appeared to be a definite association between these channels and the small regions of fat necrosis. In many instances the fine black lines either terminated directly in the saponified material or surrounded it in a close network. Frequently the saponified material was tinted gray by the infiltration of graphite particles. On gross examination a few lesions were found, especially in the perirenal fat, which were not associated with the delineated channels.

No diaphragmatic apertures between the peritoneal and the thoracic cavity were visible on gross examination.

The thoracic cavity was opened by cutting the ribs along their lateral margins and turning back the anterior portion of the thoracic wall. In no instance did the thoracic cavity contain fluid. The sternal and other mediastinal lymph channels

14. Higgins, G. M., and Murphy, G. T.: *Anat. Rec.* **40**:15, 1928.

15. Lot no. 3254675, supplied by Parke, Davis & Company, Detroit.

16. Higgins, G. M., and Graham, A. S.: *Arch. Surg.* **19**:453, 1929.

were delineated, and the mediastinal lymph nodes were black with graphite in all instances. In every rat evidence of fat necrosis was found at some point along these lymphatics. The lesions were found in the fat along the sternal channels, in the adipose tissue lying in front of the inferior venae cavae and in the fat immediately adjacent to the blackened mediastinal nodes (fig. 1 *a*).

The findings in the 9 rats that died and the 16 that were killed with ether were essentially the same.

Histologic Examination: Tissue was taken from each of the 25 animals for microscopic study. Included were sections of diaphragm and patches of fat necrosis from the peritoneal fat, the anterior thoracic wall, the fat about the inferior venae cavae and the mediastinal nodes and adjacent fat.



Fig. 1.—(*a*) The marker *A* indicates a left sternal lymph channel delineated by graphite and associated sites of fat necrosis on the posterior surface of the anterior portion of the thoracic wall; *B*, a mediastinal lymph node filled with graphite and surrounded by a zone of fat necrosis; *C*, multiple small necrotic regions in the adipose tissue about the inferior vena cava between the heart and the diaphragm. Unfortunately, the delineated lymphatic vessels were too fine to show up well in this illustration.

(*b*) A site of fat necrosis from the mesentery showing the related graphite-filled lymph channels. Under higher magnification graphite particles could also be seen infiltrating the saponified material.

Examination of many of the small regions of fat necrosis from the various peritoneal sites revealed a constant relationship between these lesions and graphite-filled lymphatics which varied in size from tiny intercellular spaces to channels easily visible macroscopically. Frequently graphite particles were also found infiltrating the saponified material of the lesions (fig. 1 *b*).

Sections of diaphragm were studied, and graphite was found in the lymph channels. The sternal lymphatics of the anterior thoracic wall were also found to

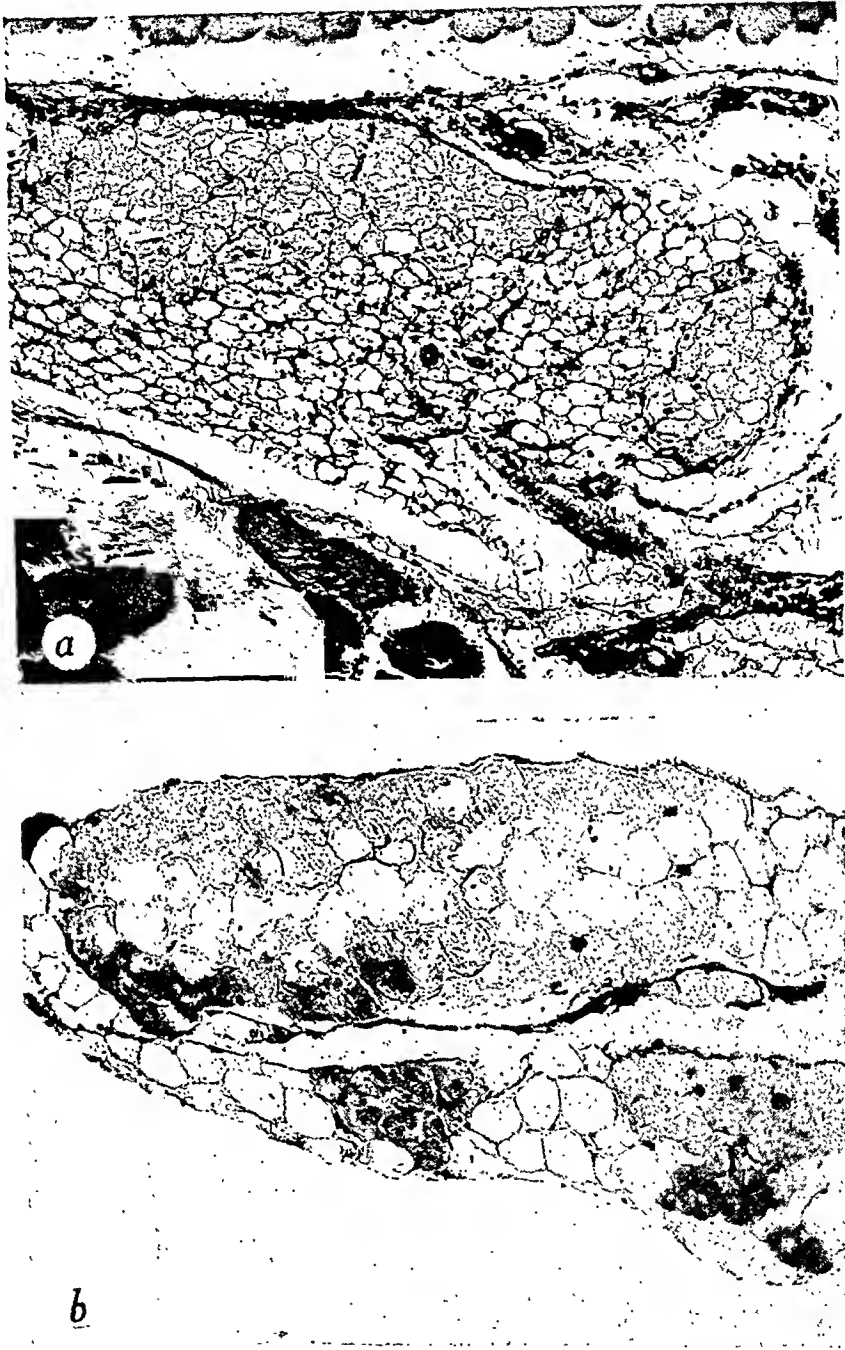


Fig. 2.—(a) Section from the anterior thoracic wall showing sites of fat necrosis associated with graphite-filled sternal lymph channels. (b) Sites of necrosis associated with graphite-filled lymph channels from the adipose tissue around the inferior vena cava.

be filled with graphite, and closely associated areas of fat necrosis were observed in the adjacent fat as illustrated in figure 2 a. A similar relationship existed between

the lymph channels and areas of necrosis in the fat about the inferior vena cavae (fig. 2 *b*). The mediastinal lymph nodes were found to be filled with both free and phagocytosed graphite particles and often necrotic areas were observed in the fat surrounding these nodes (fig. 3).

After the studies described in this paper had been completed, a case of acute pancreatitis was encountered at the Mayo Clinic. The patient died, and at necropsy evidence of abundant fat necrosis was found in both the peritoneal and the thoracic cavity. The distribution of the sites of disseminated fat necrosis was similar to that produced in the animals studied, and therefore the pertinent points of this case were included to demonstrate more conclusively that lipase is transmitted through the lymph channels.

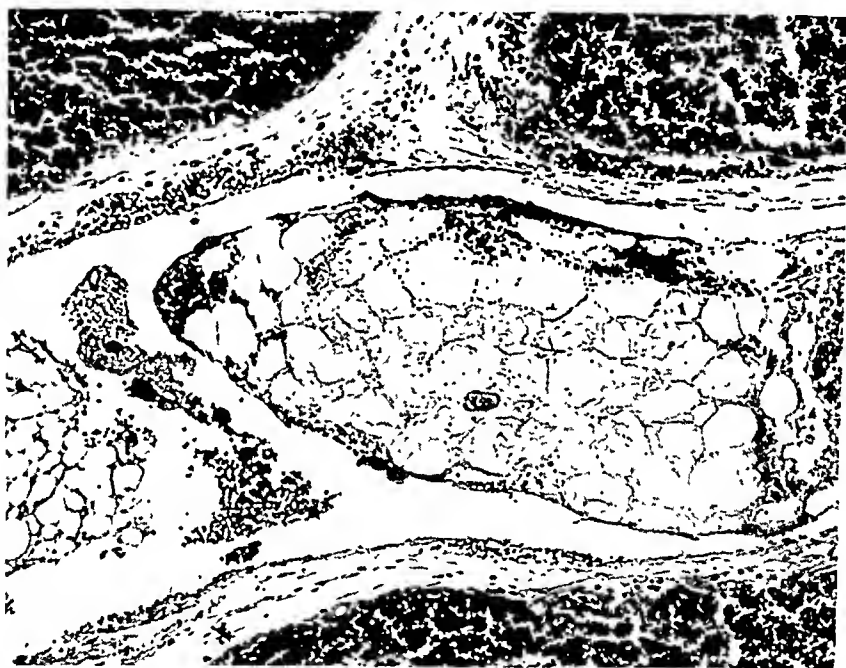


Fig. 3.—Site of fat necrosis lying between graphite-filled mediastinal lymph nodes. Higher magnification showed graphite particles within the saponified lesion.

REPORT OF A CASE

A white housewife 37 years old was admitted to the clinic and was hospitalized immediately. She had had recurrent attacks of severe epigastric pain and pain in the lower part of the thorax. Exploratory operation was carried out because it was suspected that a stone was present in the common bile duct. The patient failed rapidly after operation, however, and died on the third postoperative day.

At necropsy 500 cc. of blood-tinged fluid was found in the peritoneal cavity. Numerous small regions of fat necrosis were found on the surface of the pancreas and in the peripancreatic fat. Evidence of fat necrosis was also present in the omentum, the mesenteries, at the hilus of the liver and that of the spleen and along the lesser curvature of the stomach. The anterior surface of the head of the pancreas presented a region (6 by 2 by 3 cm.) in which hemorrhagic pancreatic necrosis had occurred.

The pleural cavities contained no excess of fluid. Sites of fat necrosis were found scattered over the pleural surface of the diaphragm and to a less extent in the parietal pericardial fat. Similar lesions were observed on the external surface of the thoracic portion of the esophagus.

The fat along the peritoneal surface of the diaphragm presented necrotic spots. In 1 instance the foci appeared to be distributed along the perivascular lymph channels of a small artery and vein (fig. 4*a*).

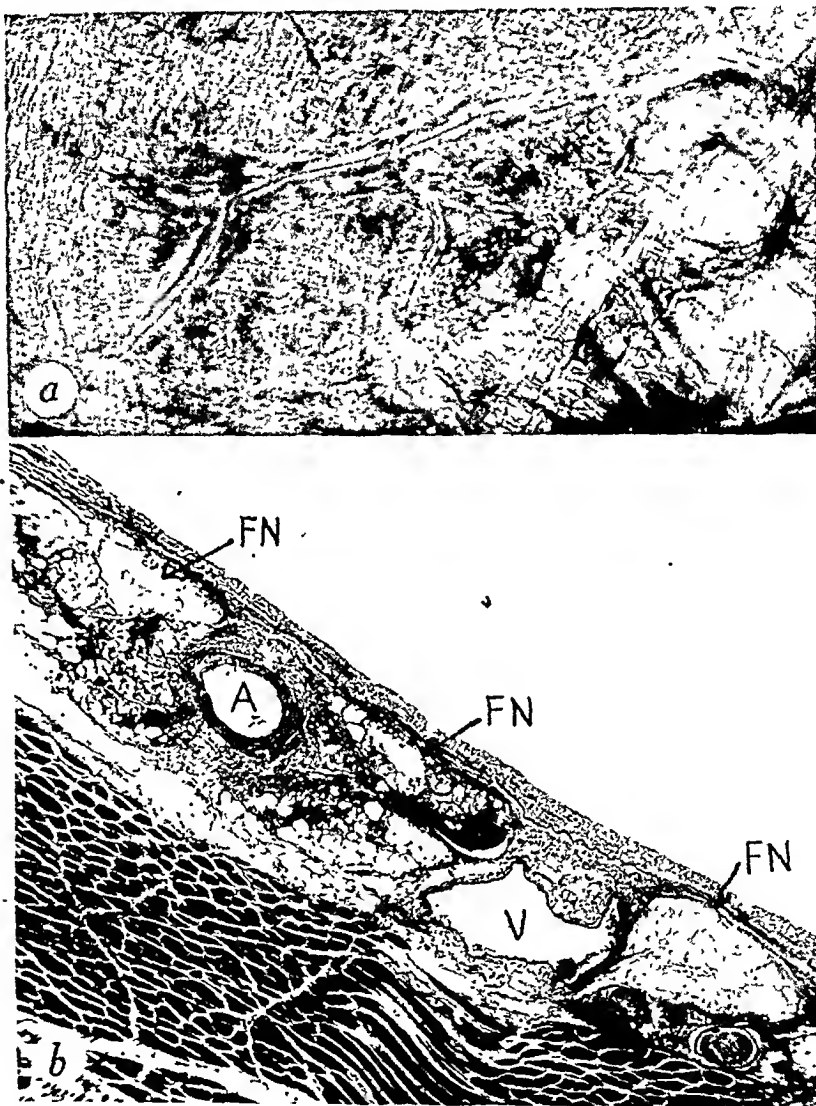


Fig. 4.—(a) Peritoneal surface of the diaphragm showing evidence of fat necrosis in the region of the perivascular lymph channels of a small artery and vein. (b) Section across small artery (A) and vein (V) shown in *a*. Typical evidence of fat necrosis (FN) may be seen in the region of the perivascular lymph channels.

Microscopic examination of sections of this portion of the diaphragm showed evidence of typical fat necrosis in the region of the perivascular lymphatics (fig. 4*b*).

The pathologic diagnosis was chronic pancreatitis with extensive fat necrosis.

COMMENT

The evidence presented in this study seems to lend strong support to the hypothesis that lipase is transmitted by means of the lymph channels in so-called pancreatic and peripancreatic fat necrosis.

It has been shown in studies of animals that intraperitoneal injection of lipolytic substances or damage of the pancreas and its duct system will cause necrosis not only in the fat of the peritoneal cavity but frequently also in the subpleural, pericardial and mediastinal fat. A similar distribution of sites of fat necrosis has been noted at necropsy in cases of pancreatic disease recorded in the literature and was seen at necropsy in 1 case at the Mayo Clinic.

Higgins and Graham demonstrated that in dogs the peritoneal and thoracic lymphatic systems are continuous by injecting intraperitoneally a suspension of finely particulate graphite. A somewhat similar lymphatic continuity was said to exist in human beings.

In this study, the graphite suspension mixed with pancreatin and injected intraperitoneally in rats showed not only continuity between the peritoneal and thoracic lymphatic channels but also a close and constant association between these delineated channels and the sites of the fat necrosis which was produced. It was possible to bring about fat necrosis in both the peritoneal and the intrathoracic fat. The sites of fat necrosis in the subpleural and mediastinal fat were not only intimately associated with the delineated lymph channels but were infiltrated by graphite particles. The mediastinal lymph nodes were consistently filled with graphite, and frequently sites of fat necrosis were observed in the immediately adjacent fat, whereas the fat a few millimeters distant was free from such lesions.

The blood vessels were examined as a possible route of the transmission of lipase. They were found to contain no graphite, and no constant association between them and the sites of fat necrosis was noted. Although it is impossible to exclude the blood vessels entirely as a factor in the transmission of lipase, it seems improbable that they were concerned.

Since the diaphragm presented no grossly visible apertures and the thoracic cavities contained no free fluid, the possibility that lipase passes directly into the thoracic cavity seems to be ruled out. Apparently, therefore, the only probable route that lipase could have taken was that through the lymph channels.

A case of chronic pancreatitis in which the associated fat necrosis was disseminated to the intrathoracic fat has been included in this study. The distribution of the sites of fat necrosis was essentially similar to that observed in the rats and it seemed difficult to explain the presence of

these lesions within the thoracic cavity except by the hypothesis that lipase was transmitted by means of the lymphatics.

SUMMARY

Intraperitoneal injections of a mixture of a solution of pancreatin and a suspension of finely particulate graphite were made in 25 large white rats. At necropsy multiple areas of fat necrosis were found in both the abdominal and the thoracic cavities, closely associated with graphite-delineated lymph channels. The hypothesis that lipase is transmitted by the lymph channels in cases of disseminated pancreatic fat necrosis following pancreatic injury and disease is given strong support.

STUDIES OF THERMAL INJURY

IV. An Exploration of the Casualty-Producing Attributes of Conflagrations; Local and Systemic Effects of General Cutaneous Exposure to Excessive Circumambient (Air) and Circumradiant Heat of Varying Duration and Intensity

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IN PREVIOUS studies¹ in this series an investigation was made of the factors which determine the extent to which the energy of environmental heat is transferred to and through the skin, of the rate at which heat is accepted by the skin and at which the temperature of the skin rises, of the time-tissue temperature thresholds at which cutaneous injury occurs and of the sequence of gross and microscopic changes that occur in the skin incident to hyperthermic injury. The skin was exposed locally and directly to a running stream of hot water as the most readily controlled means of applying heat. Although certain predictions were made as to the effects of circumambient² and circumradiant heat, no experiments were reported in which animals were actually exposed under such conditions.

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The work described in this paper was done under a contract recommended by the Committee on Medical Research, between the Office of Scientific Research and Development of the National Research Council and the President and Fellows of Harvard College, who assume no responsibility for the accuracy of the statements contained herein.

1. (a) Henriques, F. C., Jr., and Moritz, A. R.: Studies of Thermal Injury: I. The Conduction of Heat to and Through the Skin and the Temperatures Attained Therein; a Theoretical and an Experimental Study, *Am. J. Path.*, to be published. (b) Moritz, A. R., and Henriques, F. C., Jr.: Studies of Thermal Injury: II. The Relative Importance of Time and Surface Temperature in the Causation of Cutaneous Burns *Am. J. Path.*, to be published. Moritz, A. R.: Studies of Thermal Injury: III. The Pathology and Pathogenesis of Cutaneous Burns; an Experimental Study, *Am. J. Path.*, to be published.

2. Throughout this report "circumambient" is used to denote heat that is available to the skin via conduction and convection of air.

It was realized that many, if not the majority, of severe thermal casualties occurring in man, particularly those that occur incident to military operations, result from exposures in which the skin is in contact with air rather than with water. It was obviously desirable then to acquire the kind of data that would make it possible to estimate man's tolerances, both local and systemic, of thermal exposures in which heat is transferred to the body through an envelope of air and to investigate the mechanisms by which such exposures cause disability and death.

AN EXPLORATION OF THE CASUALTY-PRODUCING ATTRIBUTES OF CONFLAGRATIONS

In order to acquire a certain amount of general or orienting information concerning the thermal and chemical attributes of conflagrations, the following pilot experiments were undertaken. It was intended to observe the rate, the magnitude and the duration of the changes that occurred in the temperature, as well as the changes in the oxygen, the carbon dioxide and the carbon monoxide content of the atmosphere, incident to the burning of gasoline in closed and in ventilated spaces and to assess the relative importance of these various changes in the production of casualties.

EXPERIMENTAL PROCEDURE

Gasoline was burned in a fireproof room having a capacity of 14.4 cubic meters. The construction of the room was such that it could be either closed or ventilated at will. The fuel was poured into shallow metal pans that completely covered the floor, which measured 1.6 by 3 meters. Approximately 4 liters of gasoline was burned during each conflagration.

Method of Measuring Temperature.—In order to measure accurately the anticipated rapid changes in temperature, 40 gage spot-welded iron-constantan thermocouples were suspended in the center of the chamber, and the resulting thermoelectric potentials were amplified by means of an electronic optical bridge circuit,³ which was capable of amplifying a 1 millivolt input to a 5 milliamperere output in less than 0.2 second. Since this amplifier is a null point instrument, it was independent of all the electronic tube characteristics, of the intensity of the light beam focused on the photocell (RCA 920, split cathode) and of the input resistance of the thermocouple leads. Two such amplifiers were constructed. Two recorders were used. One was an Esterline-Angus recording ammeter (5 milliamperere full scale) with a response time of 0.5 second. The other was a General Electric photoelectric recording milliammeter with a response time of 0.2 second. Both recorders had 12 inch (30 cm.) per minute chart drives. By means of a selector switch the sensitivities of the amplifiers were usually set so that a 40 millivolt input produced full scale deflections of the recording pen.

3. Gilbert, R. W.: *Rev. Scient. Instruments* 7:41, 1936. Muller, R. H.; Garman, R. L., and Droz, M. E.; *Experimental Electronics*, New York, Prentice-Hall, Inc., 1942.

Method of Obtaining Samples of Atmosphere for Gas Analysis.—Three long tubes, each having an internal diameter of 2 mm., extended from the outside to the center of the conflagration chamber. These tubes passed through the wall at the bottom, the middle and the top of the room. Three hundred cubic centimeter samples were withdrawn as desired by attaching evacuated flasks with ground joints to the ends of these tubes. The gas samples obtained in this manner were analyzed for oxygen, carbon dioxide and carbon monoxide by means of a standard Orsat apparatus.

TEMPERATURES DEVELOPED DURING GASOLINE CONFLAGRATIONS

Unventilated Conflagrations.—In these experiments the depletion of oxygen resulted in extinction of the conflagration in about thirty seconds after ignition of the gasoline. Approximately half of the gasoline contained in each pan remained unburned. When the door was opened after the premature extinction of the fire, the room was found to be filled with dense black smoke, and there was a strong odor of gasoline.

Figure 1 *A* shows continuous records of the temperature, provided by the two thermocouples, one of which was hung midway between the floor and the ceiling in the center of the 3 meter high conflagration chamber, and the other, about 0.9 meter above the floor.

Owing to rapid convection currents, the upper thermocouple reached higher temperatures than did the lower. The sharp peaks of the temperature curve of the upper thermocouple are also due to convection currents. The average temperatures recorded by the two thermocouples over a thirty second period were approximately the same, namely, about 500 C. At the termination of the combustion the ambient temperatures fell rapidly and uniformly. The curves shown in figure 1 are typical of all experiments in which the conflagration was unventilated.

Ventilated Conflagrations.—Figure 1 *B* shows a continuous recording of the temperature of a thermocouple which was situated about 1.5 meters above the floor during a conflagration in which ventilation sufficient to maintain complete combustion was provided.

The temperatures obtained were about the same as those recorded during unventilated conflagrations. The duration of the high temperature plateau depended on the length of time that the door was left open. In the experiment in which the record shown in figure 1 *B* was made, the door was left open for fifty seconds.

PRELIMINARY OBSERVATIONS ON ANIMALS EXPOSED TO THE HEAT AND COMBUSTION PRODUCTS OF BURNING GASOLINE

Adult dogs (6 to 8 Kg.) and young pigs (7 to 12 Kg.) were exposed in various ways to burning gasoline. The animals were anesthetized by an intraperitoneal injection of pentobarbital sodium and fastened by asbestos tape to an iron frame situated in the center of the conflagration room 54 inches (1.5 meters) above the floor. The principal data pertaining to these experiments are included in table 1.

Combined Cutaneous and Respiratory Exposure.—Animals 1 and 2 were exposed to the full effects (cutaneous and respiratory) of the burning gasoline. Throughout the entire exposure of animal 1 the door of the conflagration chamber remained closed. The fire burned out in about thirty seconds, because of insuf-

ficient oxygen. The average temperature of the air surrounding the animal during this period was 320 C. The animal was allowed to breathe the atmosphere of the unventilated room for five minutes after the fire was extinguished.

Samples of the atmosphere were taken for gas analyses as soon as the fire had burned out. The mean concentration of carbon monoxide in the atmosphere was 0.8 per cent, and the concentration of oxygen was 14.6 per cent. The carbon

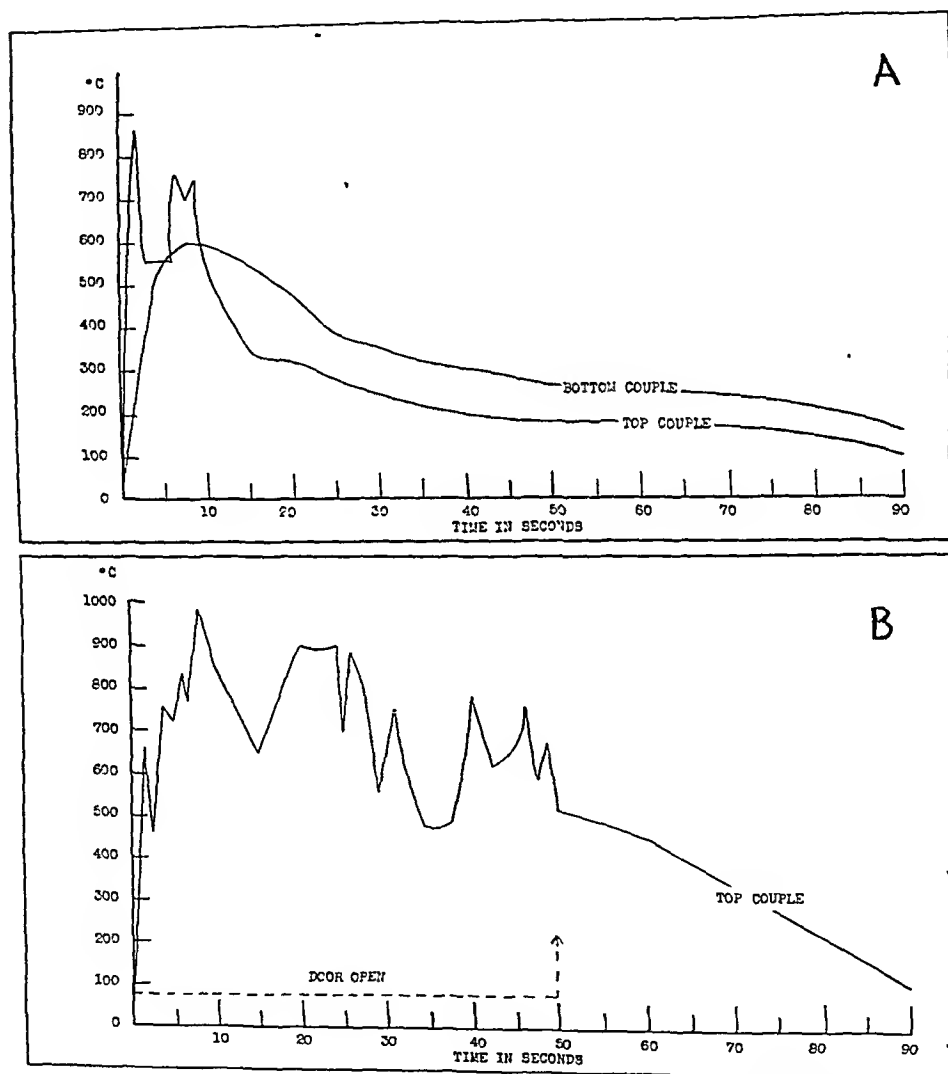


Fig. 1.—Temperature recorded continuously during burning of gasoline in a rectangular combustion chamber (6 by 10 by 10 feet [about 2 by 3, by 3 meters]). During the recording in *A* there was no ventilation of the room. Two thermocouples were used, one 5 feet (1.5 meters) and the other 3 feet (about 1 meter) above the floor level. The distance from floor to ceiling was 10 feet. During the recording in *B*, the room was ventilated for fifty seconds. One thermocouple was used, which was 5 feet above the floor.

monoxide saturation of a sample of the animal's blood taken five minutes later was 30 per cent. Although there was no indication that the fire had resulted in a dangerously low oxygen or a dangerously high carbon dioxide concentration.

it did appear likely that the animal would have died of carbon monoxide poisoning if it had remained much longer in the unventilated room.

Although animal 1 had been severely burned, it did not present early shock, required several postexposure injections of pentobarbital sodium to keep it quiet and was beginning to become restless with returning consciousness when killed six hours later. Its air passages contained an excessive amount of mucus, but there was neither clinical nor pathologic evidence of significant thermal or chemical injury of larynx, air passages or lungs, which is in accord with previous findings of Moritz, Henriques Jr. and McLean.⁴

In the case of animal 2 the door remained open during the first forty seconds of the conflagration, with the result that a larger amount of gasoline burned and a higher temperature was achieved and was maintained for a longer period than in the first experiment. At the end of forty seconds the door was closed, with the result that the fire was extinguished soon thereafter. Samples of the atmos-

TABLE 1.—*Data on Nine Experiments in Which Effects of Excessive Temperatures and of Inhalation of Combustion Products Were Observed in Animals Exposed to Gasoline Conflagrations*

Animal and Experiment No.	Conflagration		Site of Thermal Exposure			Inhalation of Combustion Products		Composition (%) of Air Immediately After Fire			Fate of Animal Blood After Exposure		
	Average Time, Sec.	Temp., C.	Body and Face	Body Only	Face Only	With Fire	After Fire	O ₂	CO ₂	CO	Dead in 15 Min.	Survival for Hrs. or Days	CO Saturation, %
1	30	320	+	+	5 min.	14.6	4.0	0.8	..	+	30
2	40	600	+	+	No	16.2	4.0	0.8	+	..	7
3	30	400	..	+	..	No	No	+	..
4	30	370	..	+	..	No	No	+	..
5	75	700	..	+	..	No	No	+
6	30	350	No	5 min.	14.7	4.6	1.0	..	+	6
7	30	350	+	+	4 min.	15.7	3.5	0.3	..	+	Trace
8	30	450	+	+	4 min.	16.1	3.6	0.7	..	+	37
9	30	500	+	+	2 min.	+	32

phere were then taken for gas analyses, and the animal was removed. This dog was moribund when removed to the open air. In view of the fact that the atmospheric concentration of carbon monoxide was similar to that observed in the preceding experiment, it was surprising to find that the carbon monoxide saturation of the blood was only 7.0 per cent. The explanation of this disparity probably lies in the fact that animal 1 breathed the atmosphere of the conflagration chamber for a total of between six and seven minutes, whereas animal 2 was moribund at the end of two minutes.

Two factors may have contributed to the extremely rapid death of dog 2. One is systemic hyperthermia caused by overheating of the blood as it circulated through the extensive superficial network of subcutaneous vessels. The other is respiratory obstruction due to pharyngeal edema. That obstruction of respiration may have contributed was indicated by the severe burning of mouth and pharynx accompanied with what appeared to be obstructive edema of the latter.

4. Moritz, A. R.; Henriques, F. C., Jr., and McLean, R.: *Am. J. Path.* **21**:311, 1945.

The trachea and bronchi contained abundant mucus mixed with carbon particles. The lungs were hyperemic.

The results of the first two experiments in which animals were exposed to burning gasoline indicated that even in circumstances that were particularly favorable to the production of carbon monoxide and to the exhaustion of oxygen, the concentration of these gases was not sufficiently altered to cause rapid asphyxia. Although the results of two experiments are not construed as evidence that neither fatal anoxia nor fatal carbon monoxide poisoning is likely to result from a gasoline fire, they do indicate that such exposures can be rapidly fatal from thermal injury alone.

Cutaneous Exposure.—The next three experiments shown in table 1 were undertaken to ascertain the effect of protecting the respiratory tract against heat and combustion products during the time that the body was being exposed. To investigate this question, animals 3, 4 and 5 wore a tight-fitting asbestos-covered mask through which a continuous stream of unheated air was circulated during their exposure to heat. The first 2 animals of this series (3 and 4) were exposed to an unventilated conflagration of about thirty seconds' duration and average atmospheric temperatures of 400 and 370 C., respectively. Although both animals showed extensive burning of the skin, they survived the immediate effects of heat and were in reasonably good condition when killed six hours later. In the case of animal 5, the door of the room was left open for the first minute of the fire, and for sixty-five seconds the temperature of the room was in excess of 400 C. Within fifteen seconds after the door was closed, the fire went out, and the animal was removed. This animal died immediately on reaching the open air and showed severe burning of all of the body surface except where the skin had been protected by the mask.

These experiments provided evidence that a relatively brief (seventy-five seconds) exposure of the skin to a sufficiently high temperature could cause almost immediate death independently of other factors.

Respiratory Exposure.—The last four experiments shown in table 1 were undertaken in an attempt to investigate further the effects produced on animals by the breathing of the combustion products of a gasoline conflagration. In each experiment the door was kept closed throughout the entire conflagration. By this procedure, postconflagration mixing of outside air with the combustion products was reduced to a minimum. The skin of the body was protected against excessive overheating by enclosing the animals to the neck in a heavy asbestos sack. With the exception of dog 6 the animals were free to breathe the burning gases and hot air during the fire as well as the smoke which remained in the chamber after the fire. Dog 6 breathed outside air circulated through the mask during the fire, and as soon as the temperature in the room had dropped to 200 C., the mask was detached by remote control and for the next five minutes only the hot smoke and air of the combustion chamber were available for respiration.

None of these 4 animals showed either clinical or pathologic evidence of thermal injury of the air passages or the lungs. Two of them (6 and 7) may have held their breath throughout most or all of the period of exposure. That animals 7 and 8 breathed during some of the time that they were in the combustion chamber is indicated by their carboxyhemoglobin concentrations of 37 and 32 per cent, respectively. It is possible, of course, that even these 2 animals held their breath during the conflagration and acquired their carbon monoxide by breathing during the interval between the time that the fire went out and

the time that they were removed from the chamber. A more extensive investigation of the problem of thermal injury of the lungs and air passages has already been reported.⁴

The most important information gained from these exploratory experiments was the observation that animals as large as dogs and pigs when exposed to this kind of conflagration for more than thirty seconds may receive injuries that are almost immediately fatal. The fatality was not necessarily contributed to by asphyxia, carbon monoxide poisoning or inhalation of flame. It was apparent that rapid death may result from systemic disturbances caused by the impact of heat energy on the surface of the body.

LOCAL AND SYSTEMIC EFFECTS IN ANIMALS WHOSE
GENERAL CUTANEOUS SURFACE WAS EXPOSED TO
CIRCUMAMBIENT (AIR) AND CIRCUMRADIANT
HEAT OF VARYING DURATION AND
INTENSITY

A series of more adequately controlled experiments was now undertaken to investigate the quantitative relationships of temperature, time and casualty production and the mechanisms responsible for casualties during general cutaneous exposure to circumambient and circumradiant heat.

EXPERIMENTAL PROCEDURE

Healthy young pigs were used. Each animal, previously clipped and anesthetized by an intraperitoneal injection of pentobarbital sodium, was fastened on a platform in the manner shown in figure 2 and a preheated oven lowered over it. In most of the experiments the snout of the pig protruded through an aperture in the bottom of the platform. There were two advantages to this method of exposure, one being that the respiratory tract was protected and the other being that it was possible to determine the time of death of any animal that succumbed during the period of exposure.

The Apparatus.—The source of heat was a bottomless oven constructed of iron and firebrick and having a capacity of approximately 1,100 liters. The box weighed 2,700 Kg., and its internal measurements were 89 by 91 by 130 cm. Four chromel alumel (10 gage) thermocouples welded onto the inside iron plates of the box provided information as to the temperature of the source of heat during the period of preexposure heating as well as during the period that the animal was being exposed. For preheating, the box was lowered into a vertical gun-annealing furnace,⁵ and when it had become thoroughly heat soaked and was at a temperature slightly higher than that to which the animal was to be exposed, it was quickly withdrawn from the furnace by an overhead crane and lowered over the platform on which the animal was suspended. The interval required for the descent of the box from the top of the tripod to the floor of the platform was between three and four seconds.

5. These facilities at the Watertown Arsenal, Watertown, Mass., were made available by the War Department.

The platform supporting the tripod on which the pig was suspended was elevated 75 cm. above the floor and was covered by a layer of dry sand. In addition to the aperture to accommodate the snout of the animal there were other openings in the platform through which wires could be passed to the temperature-recording equipment.

Three 28 gage iron-constantan thermocouples connected in parallel were fastened to the surface of the animal in such a way that the junctions were separated from the skin by a distance of between 2 and 5 cm. These provided for a continuous recording of ambient temperature.

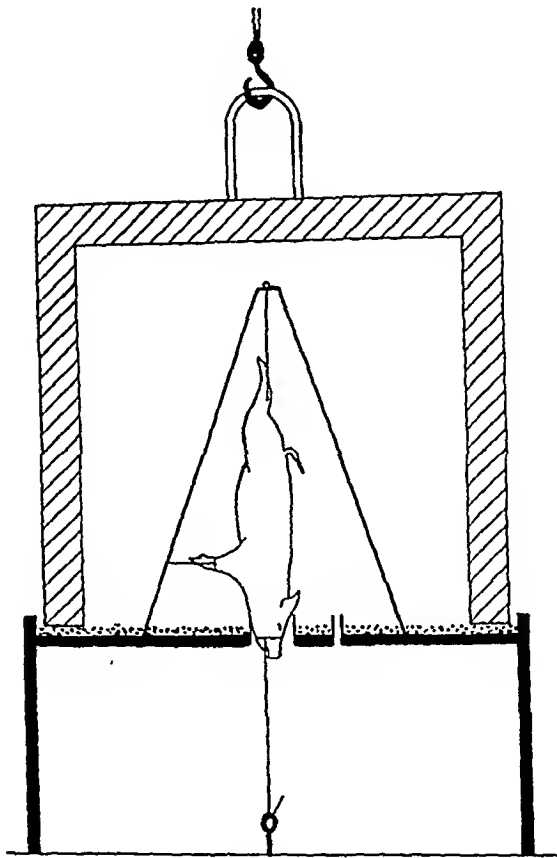


Fig. 2.—Method of exposing the experimental animal to hot air and radiant heat at Watertown Arsenal. A heavy oven constructed of iron and firebrick was preheated in a gun-annealing furnace and lowered over the platform.

Recording of Temperatures.—Rectal temperatures were taken routinely. In some experiments a rectal thermocouple provided for a continuous record. In others the temperature was taken by thermometer before and at intervals after exposure. On several occasions the postexposure temperature of the blood of the right auricle was taken for comparison with that of the rectum.

In a number of experiments a 28 gage iron-constantan thermocouple contained in a venipuncture needle was inserted into the dermis to record the temperature of the subepithelial connective tissue during and after exposure.

The temperature of the air of the exposure chamber was determined in different parts of the chamber. The values given in the text for ambient temperature

refer to the mean temperature of the air in which the animal was enveloped. The thermocouples by which the ambient temperature was measured were placed in approximately the same positions in relation to the animal in all experiments. One was fastened to the skin just below the base of the tail and one on each side of the midportion of the body. It was regularly observed that the mean ambient temperature was approximately 20 per cent lower than that measured by the thermocouples incorporated in the wall of the exposure chamber. Although the rate of cooling of the exposure chamber (and of the air contained by it) varied according to the magnitude of the initial difference between its temperature and that of the room, the drop was never in excess of 5 per cent in experiments lasting less than fifteen minutes.

On account of the convection currents that resulted from the difference between the temperature of the surface of the animal and that of the air surrounding it, the temperatures recorded in various parts of the exposure chamber showed remarkably little variation. Thus in the midhorizontal axis of the chamber there was less than 5 per cent difference in temperature between a point 15 cm. internal to the wall and a point 15 cm. external to the animal. In the midvertical axis there was less than 15 per cent difference in the temperature of the air between a point 15 cm. below the roof and a point 15 cm. above the floor of the exposure chamber.

Measurement of Heat Transfer.—Under the conditions in which these exposures were made there were three mechanisms by which heat could be transferred from the hot walls of the box to the surface of the animal, namely, air conduction, air convection and infra-red radiation. The energy transferred by conduction and convection is hereafter designated as ambient, and that transferred by radiation as radiant. Although the relative importance of these two types of heat transfer can be directly computed by means of equations 1 and 2 of a previous report^{1a} in this series, it was decided to verify those calculations under the conditions that prevailed in these experiments. Unfortunately, direct determinations of the ambient and the radiant caloric uptakes of animals were not feasible, and it was necessary to obtain these values by means of calorimeters which had been suspended in the center of the exposure chamber preliminary to animal experimentation.

The calorimeters consisted of copper cylinders which measured 2.5 cm. in diameter and 5 cm. in length. One of each pair of cylinders was gold plated, and the other was blackened with colloidal graphite (Aquadag). Thus, the former measured only ambient energy, whereas the latter determined both ambient and radiant energy.

The caloric uptake rate of the calorimeters was readily calculated from their known heat capacity and surface area and the experimentally determined rate of temperature rise as measured by an iron-constantan thermocouple soldered within the calorimeter. Owing to the discrepancy in size between these calorimeters and the pigs (about 30 by 75 cm.), it was necessary to multiply the ambient calorimetric measurements by a numerical factor⁶ equal to 0.5. Since the skin is known to be nearly a perfect black body for the radiation emitted under these experimental conditions and since the dimensions of the exposure chamber were large with respect to those of the animal, the radiant caloric measurements are directly applicable.

Actually, these data, so corrected, apply to a metallic cylinder of dimensions similar to those of a pig. In view of the fact that it has been shown that under the conditions of experimentation these data would be equally applicable to

smooth and rough and to metallic and nonmetallic surfaces,⁶ it is believed that they represent a true estimation of the caloric uptake of pig skin.

The data given in table 2 are an estimation of the radiant and ambient caloric uptake rate per square centimeter per minute of pig skin when the surface temperature is 35 C. It is obvious that during the heat exposure the surface temperature increases with time, which results in a corresponding decrease in the rate of caloric uptake. For cutaneous surface temperatures not greater than 60 C. the rates of caloric uptake are directly proportional to the difference between the temperature of the surrounding air and that of the surface of the animal. Thus, for surface temperature below 60 C. the rates of caloric uptake as a function of temperature of the surface of the skin can be computed from these data. Further examination of table 2 shows that the infra-red radiation from the inside walls of the box was the principal source of the heat energy absorbed by the animals. Under conditions that produced an air temperature of 70 C.

TABLE 2.—*Estimated Caloric Uptake of the Pig When the Temperature of the Surface of the Skin is 35 C.*

Air Temperature, C.	Caloric Uptake in Calories per Sq. Cm. per Min.			Per Cent of Total Contributed by Radiant Caloric Uptake
	Nonradiant (Ambient)	Radiant *	Total	
70	0.2	0.2	0.4	50
100	0.5	0.6	1.1	55
150	1.0	1.4	2.4	58
200	1.7	2.6	4.3	61
250	2.2	4.2	6.4	65
300	3.0	6.2	9.2	68
350	3.8	9.8	13.6	72
400	4.5	17.0	21.5	79
450	5.5	24.0	29.5	81
500	6.5	35.0	41.5	85

* Because of the difference between the air temperature and the source temperature when the animal is placed in the exposure chamber, the data concerning radiant heat refer to a source temperature 20 per cent in excess of the tabulated ambient temperature.

this contribution was 50 per cent, while at 500 C. it was 85 per cent. These percentages remained nearly invariant throughout the entire time of a given heat exposure. As previously indicated, these values for the nonradiant and the radiant contribution to the caloric uptake rate can be directly computed (equations 1 and 2)^{1a} and, if this is done, it will be found that they agree with the experimental values to within 15 per cent.

EFFECTS ON ANIMALS

The results of 71 individual exposures of pigs are shown in figure 3. It was at first intended to present in this chart only the data derived from 49 experiments in which pigs of uniform weight (7 to 18 Kg.) had their general cutaneous surface about 90 per cent exposed to heat. The additional 22 experiments included those in which large animals (in excess of 15 Kg.) were used, those in which hot air was breathed during the time that the skin was being exposed and those

6. McAdams, W. H.: Heat Transmission, New York, McGraw-Hill Book Company, Inc., 1942.

in which the animals were anesthetized after rather than before exposure. When it was found that there were no significant differences in the experimental results that could be related to the body weights of the animals (7 and 32 Kg.) or to anesthesia, it was decided to present all experimental data in one chart.

The temperature and the duration of each exposure are indicated by the position of the individual experiment on the grid. The vertical points of reference

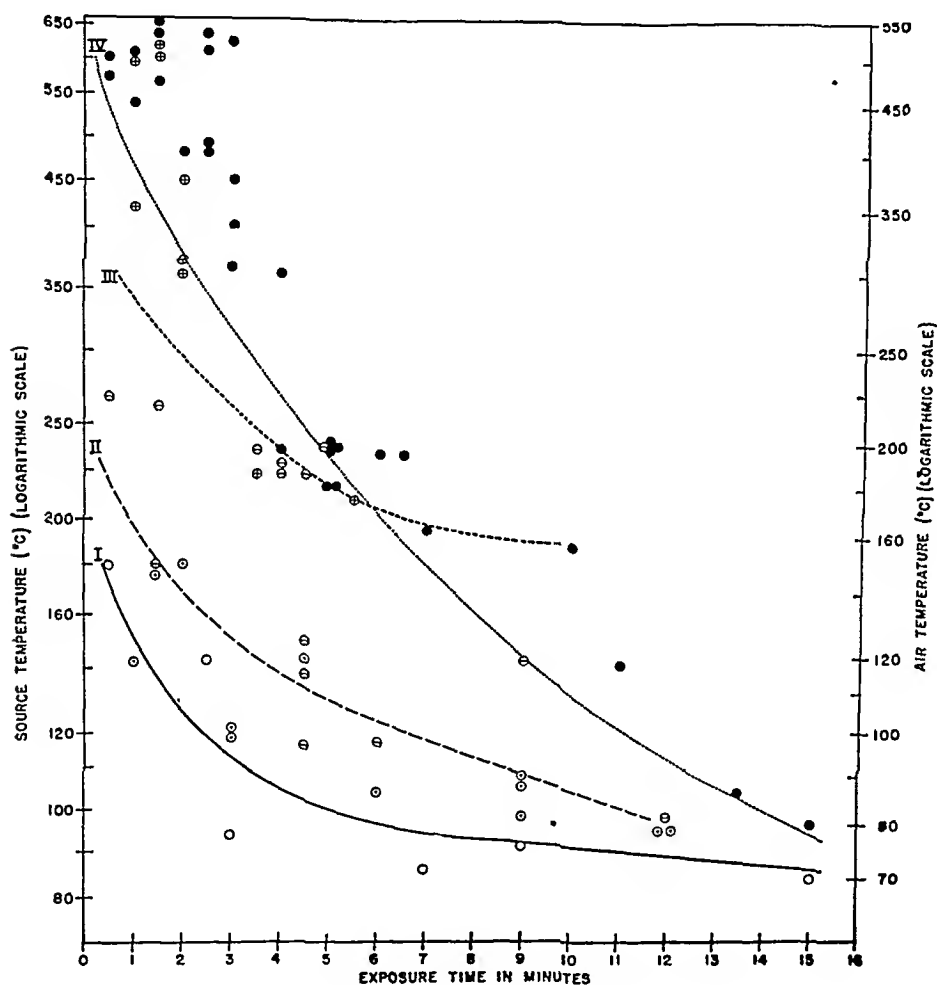


Fig. 3.—Graph showing results of 71 experiments in which pigs had their general cutaneous surface exposed to ambient and radiant heat in an oven. Each experiment is depicted by a circle. The duration and the temperature of the exposure are indicated by the position of the circle in the grid. The effect of the exposure on the pig is shown by the character of the circle. The curved lines traversing the grid depict the approximate thresholds at which varying degrees of cutaneous and systemic injury occurred.

on the left are in logarithmic progression and represent the internal surface temperature of the exposure chamber, whereas those on the right represent the corresponding ambient temperature in the vicinity (within 5 cm.) of the animal. The horizontal axis indicates the duration of the exposure.

In addition to the experiments shown in figure 3, several pigs were exposed for periods longer than fifteen minutes at source temperatures of 90 C. or higher. At 80 C. pigs survived exposures as long as forty-five minutes without cutaneous burning and without the development of fatal hyperthermia. After forty-five minutes at approximately 90 C. fatal hyperthermia developed, and although the skin was intensely hyperemic, there was no evidence that irreversible epidermal injury had been sustained.

It may be seen that the experiments fall into three main groups so far as their effects on the pigs were concerned: Some animals were unharmed and showed neither superficial nor systemic evidence of injury, others sustained cutaneous injury but with insufficient systemic disturbance to result in early collapse and death, and still others died during or within thirty minutes after the exposure.

All animals underwent a rise in body temperature as a result of exposure, and so far as the blood temperature was concerned, the maximum rise usually coincided with or was attained within a few minutes after the conclusion of the exposure. If death was to result from systemic hyperthermia, it usually occurred soon after the maximum body temperature was reached—almost invariably within thirty minutes. If an animal survived long enough after exposure for its body temperature to fall to 40 C. or lower, it characteristically survived many hours and either recovered or was killed. No example of persistent hyperthermia such as that which commonly follows heat stroke in man was encountered. The relation of systemic hyperthermia and death in pigs will be discussed in greater detail in a later section of this report.

The upper limits of exposures which pigs survived without either cutaneous burning or fatal systemic hyperthermia are indicated by the first line (*I*) that traverses the grid from left to right. Exposures lying below this line failed to cause lasting disturbances, either local or systemic. Exposures lying between the first and second lines characteristically resulted in mild or localized burning. (See figure 4*A*.) The second line (*II*) represents the approximate threshold at which generalized hyperemic burning occurred. (See figure 4*B*.) The third line (*III*) represents the approximate threshold at which the burned skin and subcutaneous tissue underwent ischemic coagulation. (See figure 4*C*.) The skin of most pigs that received exposures above this threshold was pale, and the loss of elasticity of the coagulated superficial tissues resulted in the formation of deep fissures when the extremities were flexed. The uppermost curve (*IV*), which eventually intersects the others in its descent, represents the approximate threshold at which rapidly fatal systemic hyperthermia occurred. Most pigs receiving exposures in excess of this threshold died within a few minutes after the oven had been lifted from the platform. (This period was usually under fifteen and occasionally as long as thirty minutes.)

Comparison of Effects of Hot Air and Hot Water Exposures.—In figure 5 are depicted the temperature-time relationships that were required to produce transepidermal necrosis in hot air exposure and in hot water exposure; in the latter the surface of the skin was maintained at essentially the same temperature as that of the source.^{1b} A comparison of the two curves shows that a fifteen minute exposure to water at 48 C. was sufficient to produce approximately the same degree

of injury that resulted from a fifteen minute exposure to circumambient and radiant heat at an air temperature between 75 and 80 C. A one minute exposure to hot water at 53 C. produced about the same degree of injury as resulted from a one minute exposure to ambient-and radiant heat at 160 C. It is apparent, therefore, that the surface temperatures responsible for the kind of irreversible injury observed at threshold *II* in figure 3 were considerably lower than the recorded ambient temperatures at which they were produced. In the hot water exposures the change of tissue temperature with time was determined by the rate at which

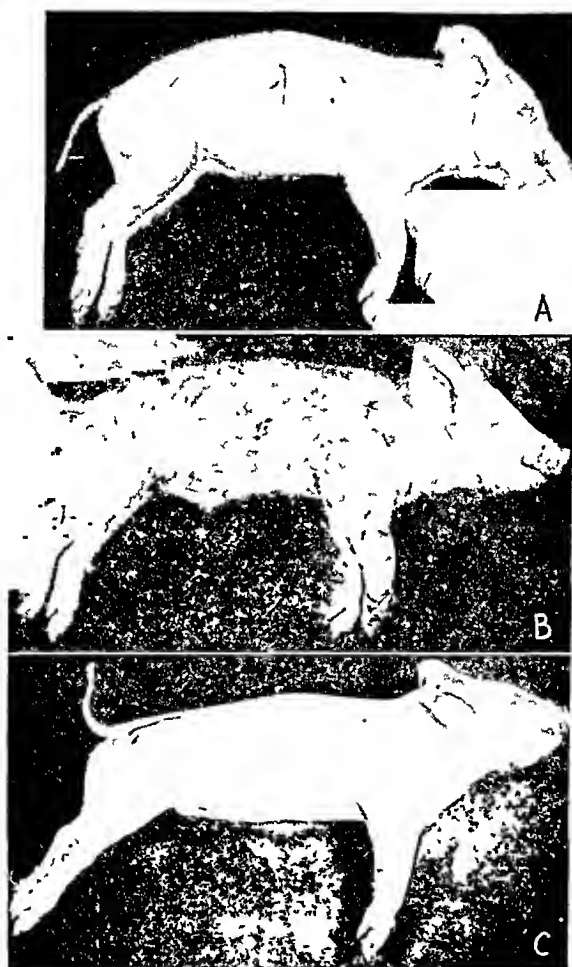


Fig. 4.—*A*, mild, localized burning of the skin of an animal that was exposed for nine minutes at an air temperature of 80 C. (See figure 3). Fatal hyperthermia resulted from an eighteen minute exposure at this temperature.

B, generalized hyperemic burning of an animal that was exposed for four minutes at an air temperature of 180 C. Fatal hyperthermia (43.5 C.) developed, and the animal died within twenty minutes.

C, generalized ischemic burning of an animal that was exposed for five minutes at an air temperature of 200 C. This animal died of systemic hyperthermia (44.5 C.) during the exposure.

heat flowed through the skin, whereas in the oven exposures it was limited by the rate at which heat was transferred to the surface.

Through the application of the general theory of heat flow, the actual time-temperature relationships within the epidermis for both hot water and hot air exposures have been computed and tabulated in a previous report (equation 6; tables 5 and 6),^{1a} and it appears that the production of a given degree of thermal injury depends only on the time-temperature relationships within the tissue, irrespective of the source of the heat. Furthermore, it is apparent in the case of circumambient and radiant heat that long before the temperature of the surface of the skin would approach that of the air the animal would have succumbed to generalized hyperthermia.

Probable Effects of Comparable Exposures on Man.—So far as the cutaneous effects of ambient and radiant heat are concerned, the reactions of man and pig should be similar if the time-temperature relationships within the epidermis were the same in each instance. (See figure 4—Henriques and Moritz.^{1b}) However, a predictable dif-

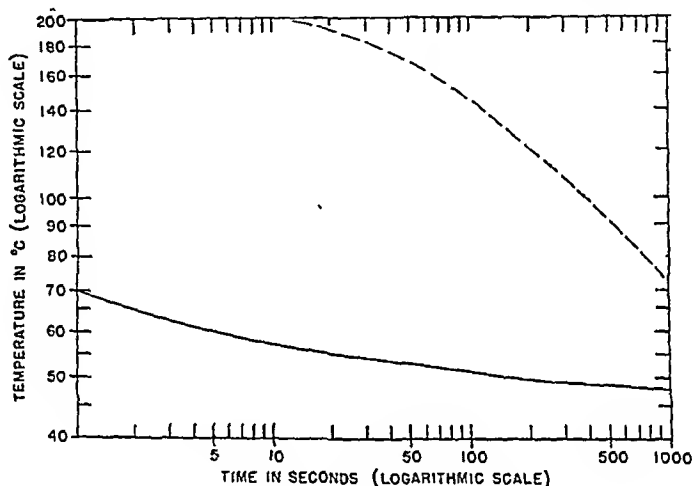


Fig. 5.—The solid curve depicts the time-source temperature relationships requisite to complete transepidermal necrosis when a cutaneous site was exposed to flowing water of constant temperature.^{1b} The broken curve shows the time-air temperature relationships (curve II of fig. 3) that produced a similar degree of injury when the cutaneous surface was surrounded by an envelope of radiant and ambient heat (oven experiments).

ference in these relationships during identical heat exposures of this type arises from the fact that sweating of human skin can undoubtedly elevate the time threshold at which cutaneous burning occurs.

That sweating can afford considerable protection in relatively low intensity hot air exposures can be assumed from the fact that man may lose moisture by this mechanism at the rate of approximately a liter per hour. This could result in heat being lost at the rate of between 0.5 and 1.0 calory per square centimeter of cutaneous surface per minute. Heat lost by porcine skin through vaporization of moisture is relatively slight (about 0.1 calory per square centimeter per minute^{1b}). Thus, in view of the data concerning caloric uptake (table 2) it seems possible that the time threshold for cutaneous burning is appreciably higher for man

than for the pig at all circumambient and radiant temperatures lower than about 120 C. That such a degree of protection would be afforded at higher air temperatures is unlikely since it would be necessary to assume that sweating was already established at a significant level at the moment of exposure and that all of the sweat excreted was vaporized. No experiments were conducted to establish the quantitative extent to which sweating may be capable of protecting human skin against thermal injury at either low or high ambient and/or radiant temperatures.

It should be emphasized that these data refer only to unclothed animals. It is possible to estimate the degree of protection afforded by articles of clothing by a knowledge of their impedance of the heat reaching the surface of the skin,⁶ but since this thermal impedance is so dependent on the physical characteristics of the fabrics involved, on tightness of fit and on the type of heat exposure, further consideration of this problem is not warranted.

*Death of Pigs*⁷.—It may be seen from figure 3 that rapidly fatal physiologic disturbances resulted from a wide range of thermal exposures and that at any given temperature within the range investigated survival or death was determined by the duration of the exposure. Observations were made on the various pathologic and physiologic changes resulting from sublethal and lethal cutaneous exposures to heat.

Pathologic Changes.—There was no apparent relationship between the occurrence of early death and the severity of the cutaneous injury. Some animals that died during or soon after exposure at relatively low temperatures showed remarkably little evidence of cutaneous injury. Others that received extensive third degree burns at higher temperatures survived many hours after exposure and showed no signs of impending death at the time at which they were killed. It was obvious that cutaneous injury per se was not responsible for early collapse and death.

Apart from cutaneous burning there were no significant differences in the pathologic changes observed in animals that died after being exposed to high temperatures for short periods and in those that died after being exposed to lower temperatures for longer periods. The pathologic characteristics of these and other cutaneous burns have been described in detail in another ^{1c} of this series of reports.

The most constant postmortem finding in all animals that died of hyperthermic shock within thirty minutes after exposure was the small and large foci of hemorrhage disseminated throughout the internal viscera. These were seen most frequently and prominently beneath the endocardium of the right and the left ventricle.

7. Several goats and dogs received exposures estimated to be lethal or sublethal for pigs, and the impression was gained that their susceptibility to fatal systemic hyperthermia did not differ significantly from that of the pig.

The right auricle was characteristically dilated and filled with dark red, unclotted blood. The impression was gained that the ventricles were more frequently found in the state of contraction after high than after low intensity exposures.

Even in pigs that breathed air so hot that their nasal mucosa was charred, there was no evidence of injury to their bronchial mucosa or lungs. The lungs of pigs rarely showed more than a mild degree of edema, irrespective of the type or the severity of exposure. The moderate to severe pulmonary edema that was observed in dogs and goats bore no relation to the inhalation of heat but was a result of circulatory failure incident to systemic hyperthermia. Fat emboli were usually present in the pulmonary capillaries but not in numbers such as to constitute an adequate explanation of death.

Animals killed twelve to twenty-four hours after sustaining severe cutaneous burns frequently showed severe parenchymatous degeneration of the adrenal cortex, the liver and the renal tubular epithelium. Neither hematoxylin and eosin nor Nissl preparation of the brains of animals dead of hyperthermia disclosed evidence of degenerative or reactive change. It should be borne in mind, however, that hemoglobin casts were sometimes observed in the collecting tubules of the kidneys and that the urine of animals regularly contained large amounts of blood pigment if severe burning was survived by more than a few hours.

Changes in Blood.—Erythrocytes: Examination of severely burned animals regularly showed intravascular hemolysis. That hemolysis was not the determining factor in survival was indicated by its absence in animals that died of hyperthermia following low intensity exposures. A more complete discussion of the relationship between intensity of thermal exposure and hemolysis will be found in number VI of this series of reports.⁸

Examination of wet and dry smears of blood of severely burned animals disclosed microspherocytosis and disintegration of erythrocytes. (See figure 6.) These changes were similar to those observed in the blood of burned human subjects by Shen, Ham and Fleming.⁹ They were not observed in the blood of animals that died after low intensity thermal exposures. In severely burned animals there was an increase both in the clotting time and in the fragility of erythrocytes.

Plasma: The observation of turbidity of the plasma together with the findings of small agglomerates of protein and enmeshed cells in wet

8. McLean, R.; Moritz, A. R., and Roos, A.: Studies of Thermal Injury: VI. Hyperpotassemia Caused by Cutaneous Exposure to Excessive Heat, J. Clin. Investigation, to be published.

9. Shen, S. C.; Ham, T. H., and Fleming, E. M.: New England J. Med. 229:701, 1943.

smears of blood of some fatally burned animals led to a reinvestigation of a phenomenon described by Kabat and Levine.¹⁰ These observers reported that 4 cc. of heated citrated plasma intravenously injected into a cat caused immediate death. After centrifugation of such plasma they found that the supernatant fluid produced no ill effects, whereas death resulted from the intravenous injection of the suspended sediment.

A repetition of the experiments of Kabat and Levine resulted in the observation that blood pressure fell rapidly and that some animals died

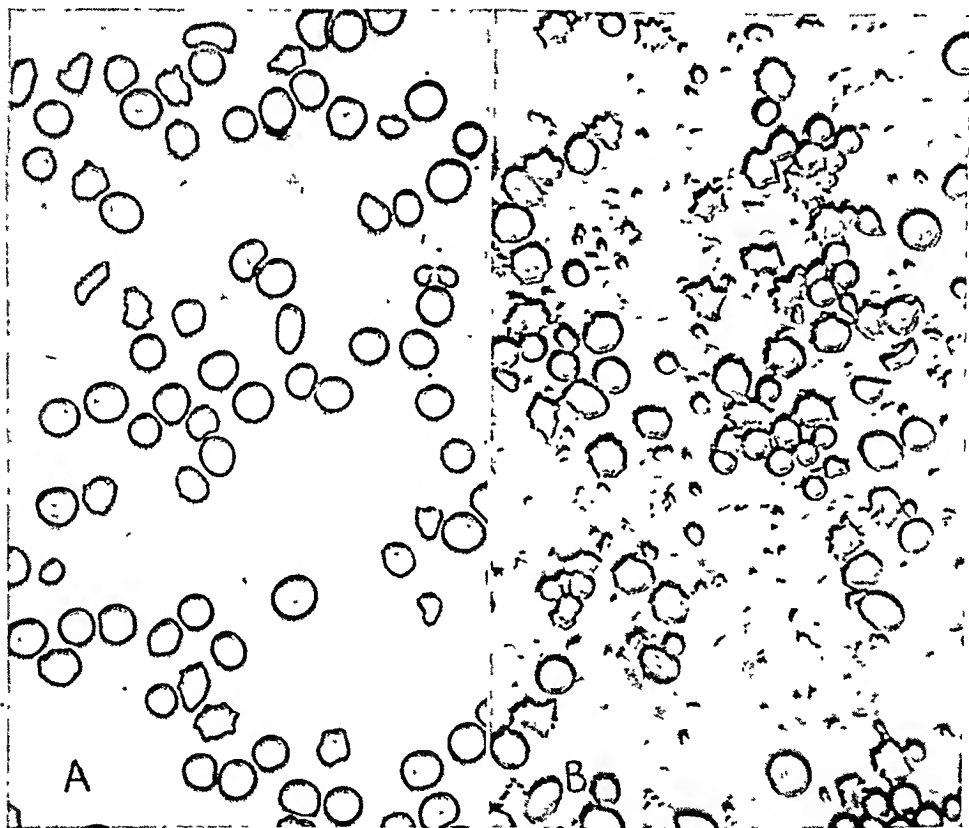


Fig. 6.—Blood smears of pig 856 (9.1 Kg.) before (A) and three minutes after (B) a five minute exposure to ambient and radiant heat at a temperature of 180 C. The animal received third degree burns of about 85 per cent of the body surface and died three minutes later with a rectal temperature of 43.5 C. Examination disclosed intravascular hemolysis, a plasma potassium concentration of 19.4 milliequivalents per liter and distintegration of erythrocytes as shown in B. During the exposure the temperature at the interface between dermis and subdermal fat, recorded by a needle thermocouple, rose to a maximum of 63 C.

in consequence of the intravenous injection of a small amount of heated citrated plasma. However, when heparin was used as anticoagulant instead of citrate, animals tolerated intravenous injection of relatively large amounts of heated plasma without ill effects and without significant

10. Kabat, H., and Levine, M.: Science 46:476, 1942.

change in blood pressure. Slight lowering of blood pressure was observed in a few animals after injection of heated heparinized plasma or of sediment of heated plasma. No deaths occurred, however, even when amounts as great as 15 cc. were used.

It was concluded that the particulate masses in preheated blood described by Kabat and Levine may be deleterious to a slight degree and in combination with sodium citrate (250 mg. per 10 cc. of blood) may cause death if injected rapidly. It is not believed, however, that these masses contributed significantly to the hyperthermic deaths observed in these experiments.

Perfusion Experiments¹¹: A heart-lung preparation (Starling method) was perfused with the blood of a dog that had died of circulatory failure seven minutes after being immersed in hot water at 70 C.

Continuous records of the heart-lung preparation included aortic pressure (in millimeters of mercury by manometer), systemic minute output (total output less coronary flow), ventricular volume (Henderson cardiometer with a Kiese volume recorder) and oxygen consumption (spirometer).

The preparation had been used earlier for a study of the metabolic effects of alloxan, and the heart was failing spontaneously at the time at which the blood from the burned animal was introduced into the perfusing system (about three hours after the preparation had been isolated). Fifty cubic centimeters of the blood of the heated animal was injected into the venous return during a period of one minute. The minute output was about 130 cc. per minute at the time of the injection, and the injected blood reached the heart diluted two or three times with the original blood of the preparation.

Six minutes later 100 cc. of the blood of the burned animal was injected again in a period of one minute. This was diluted no more than once with the blood of the preparation.

Six minutes later the blood in the venous reservoir was removed and replaced with 200 cc. of blood of the burned animal. Following this, the last, addition the heart-lung preparation was being perfused almost entirely with the blood of the heated animal.

In none of the three trials was there any significant change in the aortic pressure, the minute output, the heart rate or the oxygen consumption. Although coronary flow was not recorded, any great increase in it such as might have been expected if the blood had contained as much as 0.5 mg. of histamine would have been recognized by an increase in the discrepancy between the stroke volume as recorded by the cardiometer and the stroke volume as calculated from minute output and heart rate. Such a change was not observed.

No deleterious effect resulted from perfusing the heart-lung preparation with the blood of the burned dog. Actually there was slight evidence

11. Dr. G. K. Moe, of the department of pharmacology of Harvard Medical School, gave assistance in the conduct of this experiment.

of a beneficial effect such as would be expected from the addition of any fresh blood after three hours of perfusion.

Relation of Systemic Hyperthermia to Survival.—There appeared to be a definite correlation between survival and the height to which the internal body temperature was raised. Attention has already been called to the fact that the maximum blood and tissue temperatures were reached within a few minutes after the conclusion of an exposure and that if an animal survived for more than thirty minutes the blood temperature fell and death either did not occur or was delayed for hours or days. With few exceptions, all of the animals that died soon after exposure were found to have marked elevation of rectal temperature. In the case of exposures of long duration and low intensity the rectal temperature taken at the time of death was only slightly lower than that of the blood of the right auricle. In animals that died within a few minutes after exposures of short duration and high intensity there was characteristically a difference of several degrees between rectal and blood temperature.

The correlation between the severity of systemic hyperthermia and the occurrence of early death is shown in figure 7. With one exception, all pigs that died during the early postexposure period were those in which the rectal or heart's blood temperature rose to 42.5 C. or higher. No pig whose rectal temperature rose to 44 C. or higher survived for more than a few minutes. Eleven of the 15 animals with rectal temperatures between 43 and 44 C. and 4 of the 13 with rectal temperatures between 42 and 43 C. died during the episode of hyperthermia.

Physiologic Changes.—Prior to exposure of several pigs to hot air, asbestos-insulated electrocardiographic leads were connected with the extremities, and a carotid cannula was introduced. The effect of the exposure on the rate and the amplitude of respiration, the pulse rate, the arterial blood pressure and the conduction system of the heart was observed.

Within a few seconds after exposure there was a sharp increase both in blood pressure and in rate of respiration. The respiratory rate continued to increase and remained rapid for some time after termination of the exposure. The blood pressure soon after the initial rise fell to or slightly below the preexposure level. In some animals the pressure was well maintained at that level until within a few minutes before death, whereas in others there was a gradual and progressive decline beginning immediately at the conclusion of the initial rise.

Electrocardiographic abnormalities¹² were observed in some animals soon after the beginning of exposure, whereas in others such changes

12. Dr. Howard Eder, of the department of medicine of Harvard Medical School, took and interpreted the electrocardiograms.

did not develop until well after the onset of circulatory failure. Abnormalities observed soon after the beginning of the exposure (within two or three minutes) included increase in rate, reduction in the voltage of the QRS complex and inversion of the T waves. Ventricular extrasystoles were noted and as the exposure was prolonged there were greater disturbances in rhythm. In such animals ventricular tachycardia developed, followed by fibrillation and death.

Although abnormalities were sometimes observed in the electrocardiogram before there was evidence of respiratory failure, the terminal

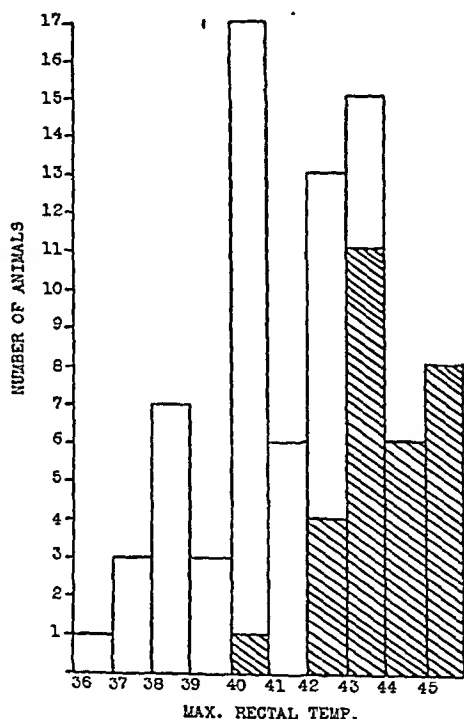


Fig. 7.—Distribution of animals according to the maximum thirty minute rise in rectal temperature following exposure to ambient and radiant heat. The initial temperatures were low because of pentobarbital sodium anesthesia. The open portions of the columns represent animals that survived; the shaded portions, animals that died during or within thirty minutes after exposure. It is apparent that there is a close correlation between systemic hyperthermia and death.

and agonal fall in blood pressure usually occurred about the same time that tachypnea gave way to intermittent periods of apnea.

The results of these experiments indicated that there are two types of hyperthermic circulatory failure, one central and the other peripheral. The former occurred in some animals as the result of brief exposures at high (over 200 C.) circumambient temperatures, whereas the latter occurred after long exposures at lower temperatures. It was obvious that further and more rigidly controlled physiologic experimentation

was required. Such studies were not feasible in the circumstances in which hot air experiments were conducted. See report VII of this series.¹³

*Changes in Blood Potassium*¹⁴.—Samples of blood were withdrawn from 4 pigs by cardiac puncture before, during and after lethal exposures to hot air. It was found that the normal concentration of the potassium of the pigs' plasma was 3 to 5 milliequivalents per liter. The post-exposure concentrations were 7.3, 10.6, 17.4 and 19.6 milliequivalents per liter, respectively. The observation that cutaneous hyperthermia was capable of causing the plasma potassium to rise to as much as 17 milliequivalents per liter suggested acute potassium poisoning as a potential cause of death. Further investigation of the importance of the release of potassium as a cause of circulatory failure and death following exposure to heat will be presented in a later report (see articles VI and VII of this series¹⁵).

SUMMARY

In a series of pilot experiments in which gasoline was burned in a fireproof chamber with and without adequate ventilation, the changes that occurred in the oxygen, carbon dioxide and carbon monoxide contents and the temperature of the air were measured and the results of exposing animals to such conflagrations were observed. The most important information gained from these pilot experiments was that large animals exposed to such conflagrations may receive injuries that are almost immediately fatal and that the fatality is not necessarily contributed to by asphyxia, carbon monoxide poisoning or inhalation of flame or fumes. It was apparent that almost immediate death may result from systemic disturbances caused by the heat flowing through the surface of the body.

The time-temperature relationships responsible for varying degrees of cutaneous injury and for acute circulatory collapse and death incident to generalized cutaneous exposure to hot air and radiant heat were determined for the pig, and the probable effects of similar exposures of man have been discussed.

Source temperatures of approximately 90 C. could be tolerated for forty-five minutes without burning, whereas at 100 C. burning occurred in twelve minutes, and at 180 C. thirty seconds was the longest exposure that could be tolerated without irreversible cutaneous injury.

13. Roos, A.; Weisiger, J. R., and Moritz, A. R.: Studies of Thermal Injury: VII. Physiological Mechanisms Responsible for Acute Circulatory Failure and Death During Cutaneous Exposure to Excessive Heat, *J. Clin. Investigation*, to be published.

14. Miss Regina McLean made the potassium analyses, which were based on the methods described by O. H. Lowry and A. B. Hastings (*J. Biol. Chem* **143**: 257, 1942).

15. McLean, Moritz and Roos.⁸ Ross, Weisiger and Moritz.¹³

It was concluded that, although the temperature of the skin was considerably lower than that of the heat source in the hot air exposures, the relationships of time and tissue temperature that were responsible for cutaneous burning in these experiments were essentially the same as those that prevailed in the previously reported hot water exposures.

At relatively low temperatures of heated air (under 120 C.) man, because of his ability to sweat, is undoubtedly less susceptible to injury than the pig. It is doubtful, however, that sweating provides a significant degree of protection at temperatures over 120 C., because at such levels the rate at which heat is transferred to the skin is considerably more rapid than the rate at which it can be dissipated by vaporization of sweat.

It should be borne in mind that the relationships of source temperature to production of injury that were established by these experiments apply only to unprotected skin and are not valid for exposures in which hair or clothing is interposed between the skin and the source of heat.

Excessive circumambient and circumradiant heat, though applied for periods as brief as thirty seconds, are capable of precipitating in pigs, dogs and goats physiologic disturbances of sufficient severity to cause death within a few minutes. The quantitative time-temperature characteristics of exposures which produce fatal systemic hyperthermia have been presented.

The severity of the physiologic disturbances that result when animals are exposed to excessive heat are frequently disproportionate to that of the cutaneous burning. Rapidly fatal systemic hyperthermia may result from long duration exposures at temperatures insufficient to cause cutaneous burning. Higher intensity exposures may cause extensive and severe cutaneous burning and yet be of too short duration to cause a significant rise in body temperature.

The severity of the immediate physiologic disturbances resulting from exposure to excessive heat appears to bear a direct relationship to the extent to which the body temperature is increased. Exposures that failed to cause the rectal temperature to rise above 42 C. rarely and those that caused it to rise as high as 44 C. invariably resulted in rapidly fatal circulatory failure. In the pig such a rise in body temperature may occur within a few minutes after cutaneous exposure to excessive heat and does not persist for more than a few hours after termination of the exposure. In animals that died within a few minutes after being exposed to excessively high environmental temperatures, the temperature of heart's blood was consistently higher than that recorded by a rectal thermometer, and the shorter the interval between the onset of exposure and death the greater was the difference.

There is no reason to believe that man and pig differ greatly in respect to the rate at which heat is transferred from the skin to the interior of the

body. If such is the case, man's susceptibility to development of rapidly fatal hyperthermia when exposed to environmental temperatures in excess of 120 C. is probably similar to that of the pig.

Apart from the cutaneous lesions, the pathologic changes observed at autopsy could not be construed as pathognomonic of thermal injury. Subendocardial ecchymoses were the most constant and striking internal abnormalities encountered in animals that died during or soon after exposure. Other characteristic postmortem findings included stasis of blood in the peripheral vascular bed, moderate dilatation of the right auricle and persistent fluidity of the blood. In the case of extensively burned animals there was intravascular hemolysis, and smears of blood disclosed fragmentation of erythrocytes. In animals that survived severe thermal exposures as long as twenty-four hours the urine was found to contain large amounts of hemoglobin and degenerative changes were frequently identified in the cells of renal tubules, the adrenal cortex and the liver.

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STUDIES OF THERMAL INJURY

V. The Predictability and the Significance of Thermally Induced Rate Processes Leading to Irreversible Epidermal Injury

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IN PREVIOUS STUDIES of the relationships that exist between time, temperature and the production of thermal cutaneous injury¹ a sufficient number of quantitative data were derived to establish the rate process inherent in the irreversible changes of epidermis injured by heat and to invite speculation as to the basic nature of these changes.

Some of the data were derived from an investigation^{1a} in which a limited area of the skin was brought immediately to and maintained at a constant predetermined temperature and which included measurements (a) of the shortest time at which a constant predetermined cutaneous surface temperature produced transepidermal necrosis and (b) the longest time at which a constant predetermined surface temperature could be tolerated without the causation of irreversible transepidermal injury. Other data were derived from experiments^{1b} in which the entire surface of the body was exposed to hot air and radiant heat, with observations of (c) the shortest time at which circumambient and circumradiant heat of measured intensity caused transepidermal necrosis. These three threshold values for epidermal injury as related to time and temperature will be hereafter referred to as thresholds A, B and C, respectively.

Because the effect which temperature has on the physical or the chemical state of matter is dependent on time, the production of the effect is known as a rate process, and it is the purpose of this communi-

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1. (a) Moritz, A. R., and Henriques, F. C., Jr.: Studies of Thermal Injury: II. The Relative Importance of Time and Surface Temperature in the Causation of Cutaneous Burns, *Am. J. Path.*, to be published. (b) Moritz, A. R.; Henriques, F. C., Jr.; Dutra, F. R., and Weisiger, J. R.: *Arch. Path.*, this issue, p. 466.

cation to show that a general expression which applies to all rate processes enables the development of a mathematical equation which quantitatively predicts these time-temperature thresholds of epidermal injury—and to evaluate so far as possible the theoretic significance of this equation.

The development and the evaluation of this equation presuppose accurate knowledge of three parameters: duration of thermal exposure, degree of epidermal injury as judged histologically and temperature attained at the basal epidermal layer during the entire hyperthermic episode—this cell layer being specifically selected, since increases of the temperature of these cells are of fundamental import in the production of cutaneous necrosis. The first two parameters are directly determinable, while the last can be accurately evaluated from the fundamental laws of the transfer of heat and the type of thermal exposure.

The theory which enables the evaluation of the time dependence of the temperature of the basal epidermal layer is considered in detail in number I of "Studies of Thermal Injury"² and is based on the excellent premise that the ratio of total tissue thickness to epidermal thickness (about 80 microns) is infinite rather than a large finite quantity—"infinite body picture."²

This theory shows that under the experimental conditions applicable to thresholds A and B the time-temperature relationship at the basal epidermal layer can be expressed by the following equations:

$$\frac{T_s - T_t}{T_s - T_o} = \frac{2}{\sqrt{\pi}} \int_0^{\frac{x}{\sqrt{t}}} e^{-x^2} dx \quad (1)$$

where

$$\gamma = \frac{L}{2\sqrt{K/C_p\rho}} = 0.15 \quad (2)$$

where

T_t = the temperature of the basal epidermal cells at the time (t) in seconds

T_s and T_o = the temperatures of the cutaneous surface during and previous to the thermal exposure, respectively

L , K , C_p and ρ = the thickness, the thermal conductivity, the heat capacity and the density, respectively, of the epidermis

By substituting the experimental values² of L , K , C_p into equation 2, a value of 0.15 can be computed for γ if an epidermal density of 0.8 Gm. per cubic centimeter is assumed.

2. Henriques, F. C., Jr., and Moritz, A. R.: Studies of Thermal Injury: I. The Conduction of Heat to and Through the Skin and the Temperatures Attained Therein; a Theoretical and an Experimental Study, *Am. J. Path.*, to be published.

The integral (equation 1) is equal to $\pi/2$ and zero when time (t) is zero and infinite, respectively, and thus after a certain interval of time, equation 1 reduces to

$$\bar{T} = T \quad (3)$$

which states that the temperature of the basal epidermal layer ultimately becomes identical with that of the cutaneous surface. Actually there will always be a small but finite temperature gradient between the surface and the basal cell layer. Fortunately, this small difference of temperature need not be considered, since these threshold A and B data are such that for all cutaneous surface temperatures above 50 C. the duration of exposure is too short to allow the epidermis to reach heat saturation and for all temperatures below 50 C. the difference (< 0.2 C.) between the steady state temperature of the basal epidermal layer² and the temperature of the cutaneous surface is trivial.² Hence equation 1 will accurately evaluate the time-temperature relationship over the range of the experimental exposure times applicable to thresholds A and B.

An expression similar to but more complicated than equation 1 has been derived for the experimental condition pertaining to threshold C. The temperatures of the basal epidermal layer have been computed and tabulated for circumambient temperatures of 80 C., 100 C., 125 C., 150 C. and 175 C., respectively (equation 6 and table 6 of number I in this series),² and these data are directly applicable to the ensuing discussion.

PREDICTABILITY OF THERMALLY INDUCED EPIDERMAL INJURY

If the reaction leading to the thermal death of epithelium conforms to that of most chemical and physical rate processes,³ it should quantitatively bear out the following equation:

$$\frac{d\Omega}{dt} = P e^{-\Delta E/R(\bar{T}+273)} \quad (4)$$

where

$d\Omega/dt$ = the rate at which Ω , an arbitrary function of epidermal injury as determined by histologic examination, is produced

T_t = the temperature in degrees Centigrade at the basal epidermal layer at the time (t) in seconds

R = the gas constant. It is equal to 2 calories per degree Centigrade per mol P and ΔE = constants evaluated from the experimental data.

Equation 4 can also be expressed as an integral equation, namely

$$\Omega = P \int_0^t e^{-\Delta E/R(\bar{T}+273)} dt \quad (5)$$

where if T_t , the time dependence of the temperature of the basal layer

3. Glasstone, S.; Laidler, K. J., and Eyring, H.: *The Theory of Rate Processes*, New York, McGraw-Hill Book Company, Inc., 1941.

of epidermis, can be considered invariant during heat exposure, equation 5 can be integrated to equation 6,

$$\Omega = P t e^{-\Delta E/R (T+273)} \quad (6)$$

where T is the steady state temperature at the basal layer. This enables one to make the numerical evaluation of P and ΔE from a limited set of experimental observations.

Thresholds A and B.—An examination of data pertaining to threshold A and the epidermal time-temperature data as given by equation 1 (see table 5 of study I in this series²) shows that for cutaneous surface temperatures less than 50 C. the hyperthermic episode is sufficiently long to enable the use of equation 6; furthermore, the cutaneous surface temperature can be substituted for the steady state temperature of the basal epidermal cells (equation 3).

Thus, by using equation 6 in this temperature range, it is possible to evaluate numerically P and ΔE by standard graphic procedures from the threshold A data, and obtain

$$\Delta E = 150,000 \text{ CALORIES PER MOL} \quad (7)$$

and

$$P = 3.1 \times 10^{98} \text{ SEC}^{-1} \quad (8)$$

This value of P depends on the arbitrary choice of the value of unity for Ω . Thus, when threshold A, complete epidermal necrosis, is reached,

$$\Omega \equiv 1 \quad (9)$$

By again making use of equation 6, a similar analysis can be made of the threshold B data. Since these data are not so complete as those used in the foregoing discussion, it is best to use the same numerical values given by equations 7 and 8 for ΔE and P , and solve for the numerical value of Ω .

These data are best represented by

$$\Omega \equiv 0.53 \quad (10)$$

when the maximum exposure time which can be tolerated without the occurrence of epidermal necrosis is reached.

Although the values given by equations 7 to 10 for P , ΔE and Ω were obtained by means of equation 6 and a restricted portion of the experimental data, they should enable one to compute these two thresholds over the entire experimental temperature range through equation 5 so long as T_t is known.

It is possible to ascertain T_t , the time dependence of the temperature of the basal epidermal layer, by means of equation 1, where its evaluation depends on two parameters, T_o and γ . An examination of this equation shows that T_t is quite insensitive to variations in T_o , the original cutaneous surface temperature, and 35 C. will be the value used.²

In view of the uncertainties² which enter into the direct experimental evaluation of γ , its numerical value, 0.15, given under equation 2 was ascertained by obtaining the best fit to the complete threshold A data.

A consideration of equations 1 and 5, together with the requisite numerical values given by equations 2, 7 and 8, shows that all of these experimental data are completely described by the following equations:

$$\Omega = 3.1 \times 10^{98} \int_0^{\tau} e^{-75,000/(\tau+273)} d\tau \quad (11)$$

where

$$\tau = \tau_s - (\tau_s - 35) \left[\frac{2}{\sqrt{\pi}} \int_0^{0.15/\sqrt{\tau}} e^{-y^2} dy \right] \quad (12)$$

Ω = the degree of injury to be expected

T_s = the surface temperature of the skin during heat exposure

T_t = the temperature of the basal epidermal layer after the time (t) in seconds has elapsed.

The numerical values of the integral of equation 12 as a function of the limits (zero and $0.15/\sqrt{t}$) are tabulated.⁴

For $\Omega > 0.5$ and $T_s < 50$ C. the time dependence of T_t can be ignored and T_t put equal to T_s ; equation 11 can then be integrated and takes the form of equation 6, which greatly facilitates the computation of Ω . With T_s higher than 50 C. and $\Omega \leq 1$ the time dependence of T_t cannot be neglected, and the evaluation of Ω requires one of the standard methods of numerical integration.⁵

This numerical determination of Ω from the two experimental parameters t and T_s enables the prediction of the degree of epidermal injury, since an $\Omega \leq 0.53$ results in a time-temperature relationship that can be tolerated without the occurrence of irreversible epidermal injury (threshold B), and $\Omega \geq 1.0$ results in a time-temperature relationship which produces complete epidermal necrosis (threshold A).

The success with which equations 11 and 12 predict these time-temperature relationships is shown in table 1.

It can be seen that in general the experimental threshold data agree excellently with those predicted, and thus the applicability of equation 4 which has been tacitly assumed throughout this section is demonstrated. In the 4 cases in which there is appreciable variance between experimental data and predictions, either the experimental data are insufficient or the duration of heat exposure was too short to preclude considerable experimental error.^{1a} Thus equations 11 and 12 probably give a more accurate estimation of thresholds A and B than the published experimental curves (fig. 4).^{1a}

4. Pierce, B. O.: A Short Table of Integrals, Boston, Ginn and Company, 1929.

5. Ford, L. R.: Differential Equations, New York, McGraw-Hill Book Company, Inc., 1933.

The numerical computations resulting from equation 6 are also included for comparative purposes. For reasons previously stated, there is no appreciable difference between this equation and equation 11 for all cutaneous surface temperatures below 50 C. under these specific experimental conditions. Equation 6 corresponds to an experimental condition in which the basal epidermal layer is brought immediately to and maintained at a constant temperature. If this were feasible at 70 C., complete epidermal necrosis would result in three ten-thousandths of a

TABLE 1.—*Predicted Time-Temperature Thresholds of Epidermal Injury Compared with Those Experimentally Observed (The Data are Valid Only for a Thermal Exposure in Which the Surface of the Skin is Brought Immediately to and Maintained at a Constant Predetermined Temperature)*

Minimum Exposure in Seconds Resulting in Complete Trans- epidermal Necrosis (Threshold A) $\Omega \equiv 1$			Cutaneous Surface Exposure Temperature in Degrees Centigrade	Maximum Exposure in Seconds Resulting in Reversible Epidermal Injury (Threshold B) $\Omega \equiv 0.53$		
Equation 6*	Equations 11 and 12	Experimental Threshold A†		Experimental Threshold B‡	Equations 11 and 12	Equation 6*
23,000	23,000	25,000	44	18,000§	12,000	12,000
11,000	11,000	11,000	45	7,200§	5,900	5,800
5,100	5,200	5,000	46	3,000	2,800	2,700
2,400	2,500	2,400	47	1,300	1,350	1,300
1,100	1,200	1,100	48	560	650	600
580	630	570	49	260	340	310
270	325	300	50	130	165	140
130	165	160	51	75	90	68
65	91	90	52	44	52	35
16	31	35	54	18	19	8
4.4	13	16	56	8.3	8.1	2.3
0.25	3.0	5	60	2.6§	2.3	0.13
0.009	1.0	2§	65	1.0§	0.7	0.005
0.0003	0.5	1§	70	0.4	0.0002

* Above 50 C. equation 6 has no experimental significance.

† The data are reported in number II of "Studies of Thermal Injury" (see fig. 4).^{1a}

§ The experimental value is uncertain.

second. The two thousand fold difference between this value and the five-tenths second predicted by equations 11 and 12 indicates the extreme importance of the thermal capacity and conductance of the skin during the early period of heat exposure.

These tabulated values resulting from the solution of equation 11 are, of course, valid only under specific experimental conditions, namely, when the cutaneous surface temperature is immediately brought to and maintained at a constant value during the entire heat exposure.

However, equation 11 should accurately predict the degree of epidermal injury to be expected from all conceivable kinds of heat exposures so long as the temperature dependence of the cutaneous surface temperature as a function of time can be evaluated since the time-temperature relationship at the basal epidermal layer can always

be determined by means of the "infinite body" heat theory⁶ implicit in equation 12. The validity of this generalization is demonstrated in the next section, where the experimental threshold C data are compared with the values predicted by means of equation 11.

Threshold C.—In order to presage these data from equation 11, T_t , the time dependence of the temperature of the basal epidermal layer, must be evaluated under conditions in which the entire cutaneous surface is exposed to a constant source of circumambient and circumradiant heat. Values of T_t as a function of the time of thermal exposure under the requisite experimental conditions^{1b} have been computed and are tabulated in number I (table 6) of this series.²

The predicted threshold data, which were computed by substituting these basal temperatures into equation 11 and then integrating graphi-

TABLE 2.—*A Comparison of the Threshold C Data with Those Predicted
(The Data are Valid Only for Circumambient and
Circumradiant Heat Exposures.)*

Air Temperature in Degrees Centigrade	Minimum Exposure Time in Seconds Resulting in Complete Transepidermal Necrosis	
	Experimental Threshold C	Threshold C Predicted by Equation 11
80.....	700	900
100.....	300	400
125.....	200	150
150.....	85	80
175.....	35	45

cally until Ω equaled 1, are compared with those determined experimentally in table 2; comparisons were made at five air temperatures ranging from 80 to 175 C., which bounded the entire experimental range.

In view of the limited experimental data on which threshold C is based^{1b} and the uncertainty of the temperatures of the basal epidermal layer as computed under these experimental conditions,² the concordance of the experimental and the predicted values is excellent; thus, considerable confidence can be placed in the statements of the last paragraph in the previous subsection.

THE NATURE OF THE CHANGES INDUCED IN EPIDERMIS BY HYPERTHERMIA

In the previous section it was shown that the degree of epidermal injury resulting from a thermal exposure can be quantitatively predicted by the standard expression for a rate process, specifically equation 4.

6. Carslaw, H. S.: *Mathematical Theory of Conduction of Heat in Solids*, New York, The Macmillan Company, 1921. Henriques and Moritz.²

In this equation there appear two empiric and experimentally determinable constants, namely, P and ΔE ; any theoretic consideration of the cause of thermally induced transepidermal necrosis should take into account at least qualitatively the numerical values of these quantities. Aside from certain general conclusions regarding the entropy of the over-all process,³ little specific information can be obtained from the numerical value of P , since this constant is intimately connected with the as yet unknown detailed physical and chemical properties and functions of the epidermal constituents. This is not the case, however, with ΔE , and thus, before proceeding further, a brief general consideration of the nature of ΔE , the activation energy in calories per mol, is in order.

*Thermal Injury and Energy of Activation.*³—In general, the kinetics of any given physical and/or chemical process depends on the total energy content of the constituents involved. If this energy content is less than a certain critical value, known as the activation energy, the process cannot take place, and if the energy content is greater than this critical value, the process may take place; thus, the rate of the process will be proportional to the fraction of these constituents which, collectively considered, possess an energy content at least equal to the activation energy. This fraction is deduced from the Maxwell-Boltzmann energy distribution law, which states that³

$$f = e^{-\Delta E/R} \quad (13)$$

where f is this fraction. The remaining symbols have been previously defined. Equation 13 determines only the temperature coefficient of a rate process, since, as shown by equation 4, the rate of a process is also proportional to one other factor that is essentially nondependent on temperature, namely, P .

Thus, the rate of any conceivable process that may result in cell death, whatever it may be, depends on a critical energy content for the participants. The fraction of the participants, collectively considered, having this energy is determined by the activation energy and the temperature (equation 13); however, the availability of this fraction is requisite but not in itself sufficient to allow the process to proceed.

An inspection of equation 13 shows that the temperature coefficient of any kinetic process is a strong function of the activation energy; for example, in the neighborhood of 50 C. the rate of a process with an activation of 1, 10 or 100 kilocalories per mol will be altered by about 0.4, 7 or 70 per cent, respectively, per unit change of temperature in degrees centigrade.

The kinetics of a considerable number of physical and chemical phenomena has been studied in detail, and it is possible to classify all rate processes, and hence, in particular, those mechanisms which may

be of considerable importance in the general consideration of thermal injury, according to the order of magnitude of their activation energy.

During the past fifty years, numerous theories⁷ have been proposed to explain injuries thermally induced in living organisms. Before applying the aforementioned criteria to the mechanisms involved in these theories, it is necessary to characterize briefly the attributes of a living cell.⁸

The living cell appears to consist of a semirigid, relatively nonsoluble framework (e. g., nucleus, nuclear wall and cell wall) that is primarily protein in nature. This aggregate is bathed in an aqueous intracellular fluid which contains both particulate (e. g., micelles) and soluble constituents, which range from simple ions to cytoplasmic proteins of extraordinary complexity. Aside from having certain purely physical attributes (e. g., permeability, contractibility, elasticity, cohesiveness, rigidity and tensile strength), this protoplasmic entity respire, excretes, synthesizes all imaginable types of molecules, utilizes and liberates energy, and reproduces in a manner that perpetuates its own kind. This exceedingly complex metabolic activity is apparently both catalyzed and precisely controlled by a multiplicity of enzymatic proteins and functionally allied molecules which contribute to both the structural framework and the cytoplasmic fluid.

In view of this complex picture, no theory of thermal injury can be considered tenable unless it takes into account these completely integrated and precisely balanced phenomena which, taken as a whole, comprise cell life. Unfortunately, as yet, knowledge of these phenomena is meager and is limited to isolated observations on living protoplasm (e. g., cell respiration, mitosis, diffusion of a few substances through cell walls) and to certain chemical and physical properties and functions of a few of the molecules that can be extracted in a presumably unaltered state from dead cell brei.

Nevertheless, even on the basis of this limited information, it is interesting to speculate in regard to the general kinds of mechanisms that may be of importance in explaining the quantitative time-temperature relationship that results in irreversible epidermal injury as judged morphologically. These data¹ concerning injury showed that episodes of transepidermal injury can be quantitatively predicted by a rate equation with an activation energy of 150 kilocalories per mol (equation 7) over the entire experimental temperature range (cutaneous surface temperatures from 44 to 70 C. and circumambient temperatures from 80 to 175 C.).

7. Belehradek, J.: *Temperature and Living Matter*, Berlin, Verlag von Gebrüder Borntraeger, 1935.

8. Cattell, J.: *Biological Symposia*. Lancaster, Pa., Jaques Cattell Press, 1943, vol. 10.

The theories⁷ that have been advanced to explain thermal injury may be classified into three general groups:

1. Thermal alterations in proteins.

In view of the many varied functions of the proteins that contribute to the maintenance of normal cell life, it is obvious that even minor thermally induced alterations of these molecules may result in profound irreversible injuries. For example, these thermal protein changes could produce increased permeability of the nuclear and/or the cell wall, structural alterations in the nucleus itself, disintegration of the protein mitochondria present in the cytoplasm and inactivation of enzymes.

Many quantitative studies⁹ have been made on the effects of temperature on proteins, and alterations that proceed at a measurable rate between 0 and 100 C. with activation energies in excess of 50 kilocalories per mol are not unusual. The heat inactivation of invertase ($\Delta E = 110$ kilocalories at p_H 4 and $\Delta E = 52$ kilocalories at p_H 5.7) and of peroxidase ($\Delta E = 189$ kilocalories) and the heat denaturation of egg albumin ($\Delta E = 132$ kilocalories at p_H 5) and of hemoglobin ($\Delta E = 76$ kilocalories at p_H 6.8) are a few of the many examples.

Thus, the morphologic observations of protein dissolution and/or coagulation on which the quantitative judgment of transepidermal necrosis is based may well be directly due to the thermal alterations of as yet unknown proteins present in epidermal cells.

2. Other possible alterations in metabolic processes.

Since temperature affects to a greater or a lesser degree the kinetics and the thermodynamics of all chemical and physical phenomena, heat may cause alterations in metabolism irrespective of its effect on proteins. For example, the entire metabolic equilibrium may be upset if the concentrations of some of the individual constituents have changed as a result of temperature-induced variations in the rates of diffusion, formation and degradation of the chemical reactants comprised in the process; in fact, owing to this abnormal functioning, certain metabolites normally present may completely disappear and/or others abnormal and toxic in character may arise. There can be no doubt that these phenomena take place and that they may cause cell death.

Many of these metabolic reactions,^{9a, b} both enzyme and nonenzyme catalyzed, have been studied as *in vitro* processes, and activation energies usually between 10 and 20 kilocalories per mol are generally found. In certain instances the activation energies are less than 10 kilocalories per mol, but none have been found to exceed 50 kilocalories.

To date there is no experimental evidence that these types of reactions can lead to a temperature coefficient for thermal injury which corresponds to that found experimentally for transepidermal necrosis.

9. (a) Sumner, J. B., and Somers, G. F.: *Chemistry and Methods of Enzymes*, New York, Academic Press, Inc., 1943. (b) Bull, H. B.: *Physical Biochemistry*, New York, John Wiley & Sons, Inc., 1943. (c) Glasstone and others.³

3. Nonprotein induced alterations in the physical characteristics of cells.

Into this group are placed all physical phenomena that are characteristic of protoplasm but are not primarily affected by the thermal alterations of proteins contained therein. For example, diffusion of metabolites through a cell wall that has not undergone chemical alteration is a member of this class, while changes in diffusion rates that are the result of an increased permeability of cell wall due to degradation of the structural protein are specifically excluded, since this phenomenon is classified under group 1.

All of the biophysical rate processes that have been studied, such as diffusion through liquids and membranes, changes in viscosity, rigidity or tensile strength, and liquefaction, possess activation energies that are usually less than 5 kilocalories per mol and never in excess of 15 kilocalories per mol.

Although these types of mechanisms are undoubtedly potentially capable of causing cell death, they are not the instigators of the morphologic changes that are observed in irreversible epidermal injury.

Since many fatlike substances are known to melt around 45 C., the liquefaction of such substances has received some consideration as a potential instigator of thermal injury.⁷ From a kinetic standpoint the rate of melting is a physical process with activation energy essentially zero. This theory would predict that there would be a sharp temperature threshold for injury and that then the injury rate would become nearly a linear function of the increment in temperature above the threshold value. Hence, while liquefaction might account for the quantitative epidermal thermal relationships^{1a} at cutaneous surface temperatures between 45 and 48 C., there would be extreme variance with the experimental data at the higher cutaneous temperatures. The extent to which thermal liquefaction of lipid substances may contribute to cell death in tissues other than the epidermis was not investigated.

In view of the foregoing discussion it can be concluded that the only biokinetic phenomena known to date that can account for epidermal cell death are the thermally induced changes in protein structure which have an activation energy in the neighborhood of 150 kilocalories per mol.¹⁰ This in no way excludes the injury propensity of the innumera-

10. Crozier and his co-workers have used the concept of activation energy to interpret many life processes by means of some master reaction. This interpretation has been criticized by numerous investigators (Bull.^{9b} Heilbrunn, L. V.: *An Outline of General Physiology*, Philadelphia, W. B. Saunders Company, 1943), since it is mathematically demonstrable that the constancy or inconstancy of the activation energy is neither a necessary nor a sufficient condition to prove the respective existence or nonexistence of a specific master reaction. In Crozier's investigations, the activation energies found were usually in the neighborhood of

ble mechanisms implied earlier, but merely states that all quantitative studies made in this investigation indicate that the morphologic changes observed in the thermally altered epidermal tissue can be ascribed to these degradations of proteins.

As to the number and the kinds of proteins involved, the specific nature of thermally induced reactions and the individual rate of each protein alteration, nothing can be stated. Further, it is probable that at any given hyperthermic level any one of these numerous protein alterations is potentially capable of producing cell death.

Thermal Injury and Entropy and Free Energy of Activation.—With no intention whatsoever of inferring that the thermal effects on living protoplasm can be ascribed to the alteration of any single protein, it is of value to make for the moment this extreme oversimplification in order to interpret the significance of the numerical value of P in the empiric rate equation 4, which predicts completely the thresholds of transepidermal necrosis.

In vitro studies on both enzymatic and nonenzymatic proteins have shown that the rate of thermally induced changes is first order, and the quantity of degraded protein is given by ³

$$\ln \frac{C_0}{C_t} = \frac{k(T+273)}{h} t \left[e^{\Delta S/R} \right] \left[e^{\Delta E/R (T+273)} \right] \quad (14)$$

where

C_0 = the amount of protein originally present

C_t = the amount of unaltered protein present at the time (t) in seconds

$K(1.37 \times 10^{-16}$ ergs per degree), $h(6.55 \times 10^{-27}$ ergs-seconds) and $R(2$ calories per degree per mol) = the Boltzman, Plank and Gas constants, respectively

T = the temperature in degrees centigrade

ΔS and ΔE = the entropy and the activation energy of the rate process, respectively.

Comparing this equation with equation 6 and remembering that $\Omega \equiv 1$ for the production of transepidermal necrosis, one finds in the neighborhood of 50 C. the following numerical relationship for ΔS in calories per degree per mol.

$$\Delta S = 398 + 2.7n(\ln C_0/C_t) \quad (15)$$

where C_0/C_t is the reciprocal of that fraction of the original protein present which is still unaltered at the onset of transepidermal necrosis.

10 to 20 kilocalories, and since the great majority of biologic reactions are in this range, these criticisms are well justified. However, it is also mathematically demonstrable that no conceivable combinations of a series of reactions with activation energies within a certain bound can produce an over-all kinetic process with an activation energy out of this bound (e. g., no combination of reactions, for which the activation energies are greater than 10 kilocalories but less than 20 kilocalories, can lead to any over-all phenomena with an activation energy less than 10 kilocalories or greater than 20 kilocalories). Thus, the interpretation in the text is valid.

This equation is extraordinarily insensitive to the ratio C_o/C_t . Thus, the variation of this ratio from 100 (99 per cent protein alteration requisite for injury) to 1.01 (1 per cent alteration required), changes ΔS from 401 to 389. In view of this fact the analysis can at once be generalized to include all of the simultaneous inactivations and denaturations of the numerous protoplasmic proteins which are thermally induced and proceed with an activation energy in the neighborhood of 150 kilocalories per mol.

Thus, for the combined effect of these processes one finds that

$$\Delta S \approx 395 \text{ ENTROPY UNITS} \quad (16)$$

The free energy of activation, ΔF , can be computed from this entropy of activation and the experimental activation energy (150 kilocalories per mol, equation 10) from the following thermodynamic equation.³

$$\Delta F = \Delta E - (T + 273) \Delta S \quad (17)$$

And in the neighborhood of 50 C. one obtains

$$\Delta F \approx 22 \text{ KILOCALORIES PER MOL} \quad (18)$$

High energies of activation occurring together with large increases in entropy, which lead to free energies of activation of from 20 to 30 kilocalories in the neighborhood of 50 C., are a unique characteristic of all rates of heat denaturation of proteins and heat inactivation of enzymes which have been quantitatively studied in vitro.³

Certainly it is more than coincidence that the numerical constants of an experimental equation that quantitatively predicts thermally induced epidermal necrosis are in complete accord with the known temperature dependence of the in vitro alterations in protein structure, and thus one can place considerable confidence in the statement that thermally induced injury of living epidermal protoplasm is primarily due to changes in some of the nuclear and cytoplasmic proteins which have activation energies for thermal degradation in the neighborhood of 150,000 calories per mol.

LATENT THERMAL INJURY

In articles II and III of "Studies of Thermal Injury" the existence of latent or morphologically unrecognizable epidermal cellular injury following certain apparently harmless thermal exposures was proved by repeated application of prethreshold exposures. Furthermore, the time required for recovery after these exposures producing latent injury became longer the nearer the changes approached microscopic visibility.¹¹

11. Moritz, A. R.: Studies of Thermal Injury: III. The Pathology and Pathogenesis of Cutaneous Burns; an Experimental Study, *Am. J. Path.*, to be published. Moritz and Henriques.^{1a}

The concept of an unknown but definite fraction of certain of the cellular proteins that must be thermally altered before morphologically recognizable injury will result is in agreement with these experimental data.

During a thermal exposure that results in latent injury a noncritical fraction of these proteins are altered. At the termination of the exposure the epidermis rapidly approaches normal temperature,² and at least partial cell function is resumed. Thus, during the period of recovery the thermally altered proteins are replenished to a degree which depends in part on the length of the period of recovery and in part on the duration of the thermal exposure which produced unrecognizable injury.

SUMMARY

The experimental time-temperature relationships which determine the thresholds of epidermal injury (studies II and IV of "Studies of Thermal Injury") are subjected to mathematical analyses. Two types of thermal exposure are considered: those in which the cutaneous surface is immediately brought to and maintained at a constant temperature, and those in which the skin is exposed to a constant source of circumambient and circumradiant heat.

It is demonstrated that all of these data are quantitatively predicted by the following equation:

$$\Omega = 3.1 \times 10^{98} \int_0^t e^{-75,000/(T_t + 273)} dt \quad (11)$$

where T_t is the temperature in degrees centigrade of the basal epidermal layer at the time (t) in seconds. The values of T_t during the hyperthermic episode depend on the type of thermal exposure and are evaluated by means of equations developed in study I of "Studies of Thermal Injury."

All values of Ω less than or equal to 0.53 result in a time-temperature relationship that can be tolerated without the occurrence of irreversible epidermal injury; all those equal to or greater than 1 result in a time-temperature relationship which produces transepidermal necrosis.

A theoretic treatment of this equation shows that, of all the physical and chemical processes known to date, thermally induced epidermal injury must be due primarily to thermal changes of those proteins and enzymes whose rate of alteration corresponds to an activation energy of at least 150 kilocalories per mol and an entropy change of about 395 units. This is consistent with the known existence of a reversible phase of latent epidermal injury.

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NEPHROTOXIC ACTION OF DL-SERINE IN THE RAT

I. The Localization of the Renal Damage, the Phosphatase Activity and the Influence of Age, Sex, Time and Dose

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IN THE course of experiments dealing with the influence of dietary factors on the composition of tissue phospholipids, Fishman and Artom¹ observed a damaging effect of *dl*-serine (alpha-amino beta-hydroxypropionic acid) on the kidneys of young rats. They found that 50 to 100 mg. of *dl*-serine given by stomach tube or injected parenterally was injurious, while even the double amount was not toxic when added to the food.² The toxicity of *dl*-serine was markedly influenced by the diet given. Rats on an adequate stock diet had a lower mortality than those on a diet of 10 to 30 per cent casein, as the source of protein, and deficient in B vitamins. The renal anatomic changes were studied by Morehead, Fishman and Artom.³ Twenty-four hours after the initial administration of *dl*-serine they observed acute necrosis at the junction of the renal cortex and medulla. Even when the administration of *dl*-serine was continued for fourteen days resorption of the necrotic tissue took place and almost complete repair occurred in rats supplied a complete diet. In rats which were fed a diet deficient in B vitamins and low in protein the renal damage was more severe. Calcium deposits were marked after several days of the administration of *dl*-serine, and healing took place with considerable scar formation and resulting hypertrophy of the remaining undamaged tubules. While the addition of pure B vitamins to the experimental diets considerably reduced the mortality and the severity of the clinical symptoms,⁴ it did not influence the extent of the renal lesion.⁵ A

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1. Fishman, W. H., and Artom, C.: J. Biol. Chem. **145**:345, 1942.

2. Artom, C., and Fishman, W. H.: Proc. Soc. Exper. Biol. & Med. **57**:239, 1944.

3. Morehead, R. P.; Fishman, W. H., and Artom, C.: Am. J. Path. **21**: 803, 1945.

4. Fishman, W. H., and Artom, C.: Proc. Soc. Exper. Biol. & Med. **57**:241, 1944.

5. Morehead, R. P.; Fishman, W. H., and Artom, C.: Am. J. Path. **22**:385, 1946.

comparison of the relative toxicity of *l*-serine and of *dl*-serine led Artom, Fishman and Morehead⁶ to the conclusion that the toxic action was due to the unnatural *d*-isomer, since *l*-serine had no injurious effect. In young guinea pigs and rabbits, as well as in mature mice, comparable lesions of the kidneys were not found.⁷

In the present study, various factors concerning the nephrotoxic action of *dl*-serine in the rat were observed. The influence of age and sex was examined, as well as the minimal toxic dose and the time after which the first evidence of renal damage could be ascertained under the microscope. An attempt was also made by several methods to determine the exact location of the tubular damage. In addition, the influence of acute *dl*-serine poisoning on renal phosphatase activity was studied.

MATERIAL AND METHODS

Albino rats of the Wistar strain were used. Except in the experiments dealing with the influence of sex and age, they were male animals weighing approximately 100 Gm. They were fed either "Rockland rat diet complete" (Arcady Farms, Milling Co., Chicago) or, for a period of seven days, a protein-deficient, vitamin B complex-free diet of a composition similar to that of the diet 1 used by Fishman and Artom.¹ This diet consisted of vitamin test casein (General Biochemicals) 10 per cent, dextrin 39 per cent, sucrose 37 per cent, hydrogenated cottonseed oil ("crisco") 5 per cent, cod liver oil 5 per cent and salt mixture no. 2 U. S. P. XII 4 per cent. Each animal received only a single dose of *dl*-serine (Merck), either by stomach tube or by intraperitoneal injection. In a few instances the amino acid was injected intravenously into a tail vein. Unless otherwise stated, the animals were killed after twenty-four hours. Either 50 or 100 mg. of *dl*-serine dissolved in 3 cc. of water was given regularly except in those experiments in which the influence of the dose on the renal damage was examined. Tissues were fixed in 8 per cent solution of formaldehyde U. S. P. and stained with hematoxylin and eosin. For the demonstration of alkaline phosphatase activity, acetone-fixed tissues were stained according to Gomori's⁸ method as modified by Kabat and Furth.⁹ The slides were incubated for two to twelve hours in the buffer-phosphate mixture. No counterstain was used.

An attempt was made to localize the renal damage exactly by vital staining and by isolation of single renal units. Vital staining of the proximal convoluted tubules took place after 1 cc. of lithium-carmin solution had been injected subcutaneously on five consecutive days. Simultaneously with the last injection of this solution, *dl*-serine was given intraperitoneally, and the animal was killed twenty-four hours later. Sections of tissue fixed in solution of formaldehyde U. S. P. and acetone were slightly counterstained with Harris' hematoxylin. The isolation of single renal tubules was performed by Dr. Jean Oliver in the department of pathol-

6. Artom, C.; Fishman, W. H., and Morehead, R. P.: Proc. Soc. Exper. Biol. & Med. **60**:284, 1945.

7. Morehead, R. P.; Poe, W. D.; Williams, J. O., and Lazenby, M. E.: Am. J. Path. **22**:658, 1946.

8. Gomori, G.: Proc. Soc. Exper. Biol. & Med. **42**:23, 1939.

9. Kabat, E. A., and Furth, J.: Am. J. Path. **17**:303, 1941.

ogy of the Long Island College of Medicine, Brooklyn. This method consists essentially in isolating single nephrons from formaldehyde-fixed tissue that had been treated previously with strong hydrochloric acid and staining them in toto with a modified iron-hematoxylin procedure, ferric chloride being used as mordant.¹⁰

RESULTS

Localization of the Renal Injury.—Eighteen rats fed the complete, and 13 rats fed the synthetic diet all showed renal lesions after the administration of 100 mg. of *dl*-serine. On gross examination a small, irregularly shaped strip of grayish discoloration at the innermost portion of the cortex indicated the necrotic area. Microscopically, the changes agreed closely with those described by Morehead, Fishman and Artom.¹¹ Acute renal necrosis occurred at the junction of cortex and medulla twenty-four hours after the administration of *dl*-serine. The epithelium of many tubules in this area was almost completely necrotic (fig. 1).

The changes observed varied somewhat in severity in different fields. Casts composed of eosinophilic granular material were frequent, located at times in the convoluted, but mostly in the collecting tubules. Not all the tubules at the corticomedullary junction, however, were necrotic. Even where the damage was most extensive, occasional tubules were spared. In animals restricted to the synthetic diet the renal damage induced by *dl*-serine was more extensive. In some fields the necrosis extended into the more outlying portions of the cortex. The glomeruli were not affected.

The areas of necrosis were located predominantly in the outer strip of the outer zone of the medulla, according to the nomenclature of Peter,¹² which corresponds to the subcortical zone of Heidenhain.¹³

According to Heidenhain, the cortex can be divided into three zones. The narrow outermost zone (cortex corticis) contains no renal corpuscles. The central or glomerular zone can be divided radially into the pars radiata, containing branching collecting tubules and straight thick portions of loops, and the pars labyrinthica, containing renal corpuscles and convoluted tubules. The innermost or subcortical zone is without corpuscles and thin portions of loops. In this area are found the distal portions of the proximal convoluted tubules, portions of the ascending limbs of Henle, and collecting tubules.

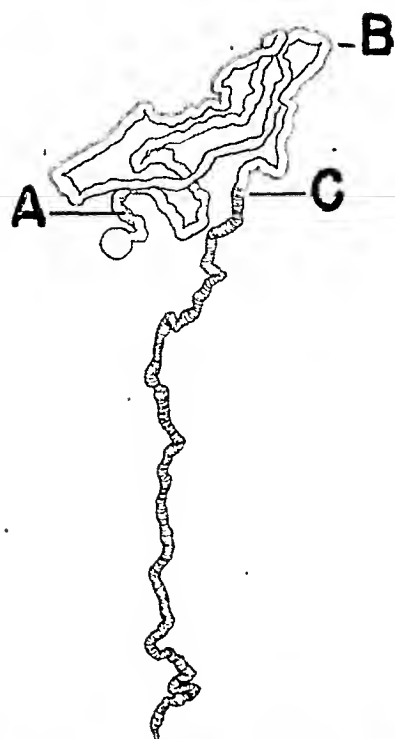
It is obvious, therefore, that the damage occurring after the administration of *dl*-serine may affect any of these three different structures.

10. Oliver, J., in Harvey Lectures, 1944-1945, Lancaster, Pa., Science Press Printing Company, 1945, vol. 40, p. 102.

11. Morehead, Fishman and Artom (footnotes 3 and 5).

12. Peter, K.: Untersuchungen über Bau und Entwicklung der Niere, Jena, Gustav Fischer, 1927, pp. 338 and 567.

13. Heidenhain, M.: Synthetische Morphologie der Niere des Menschen, Leiden, E. J. Brill, 1937.



2



Figures 1 to 4

(See legend on opposite page)

No definite decision could be made on the basis of sections stained with hematoxylin and eosin, although wherever damage was extensive the probability seemed strong that both ascending limbs of Henle's loop, as well as proximal convoluted tubules, were affected. In order to study this question further, a number of rats were given injections of lithium-carmin solution, as previously stated. Suzuki¹⁴ introduced this method for the localization of tubular injury. Only the epithelium of the proximal convoluted tubules is able to concentrate the dye in particulate form. Dye particles are found in large amounts forming coarse granules in the uppermost segment of the proximal convoluted tubules. The cells of the median portion are less intensely stained, while the cells of the distal third contain only irregularly distributed dustlike dye particles. Using this method, Suzuki was able to show that uranium and mercury affected predominantly the distal portions of the proximal convoluted tubules, while chromium damaged the proximal portion. However, the application of this method was not quite satisfactory for the present problem. In normal control rats, five injections of lithium-carmin solution gave satisfactory staining in the proximal convoluted tubules located in the outer portion of the cortex. The distal portions of the proximal convoluted tubules in the subcortical zone showed only irregular and weak staining. In the animals previously treated with carmin and subsequently given *dl*-serine, a number of the necrotic tubules showed faint diffuse carmin staining. However, this was not observed regularly, and there was considerable variation in different animals. The only conclusion that could be drawn from these experiments was that distal portions of the

14. Suzuki, T.: *Zur Morphologie der Nierensecretion unter physiologischen und pathologischen Bedingungen*, Jena, Gustav Fischer, 1912.

EXPLANATION OF FIGURES 1 TO 4

Fig. 1.—Section of the kidney of a male rat fed the synthetic diet and killed twenty-four hours after administration of 100 mg. of *dl*-serine by stomach tube. There is extensive necrosis of tubular epithelium in the subcortical zone. Hematoxylin and eosin; $\times 75$.

Fig. 2.—Camera lucida tracing of a proximal convolution isolated from the kidney of the rat furnishing the section shown in figure 1. *A*, scattered fine droplets between nuclei in the first third of the proximal convoluted tubule. The mitochondria are well preserved. *B*, beginning of the terminal segment of the proximal convolution. *C*, abrupt beginning of complete necrosis of the lower terminal segment. $\times 32$.

Fig. 3.—Section of the kidney of a male rat fed the synthetic diet and killed thirty minutes after an intravenous injection of 100 mg. of *dl*-serine. Focal tubular necrosis may be seen. Hematoxylin and eosin; $\times 120$.

Fig. 4.—A histotechnical preparation for the demonstration of alkaline phosphatase in the kidney of a rat killed twenty-four hours after an intraperitoneal injection of 50 mg. of *dl*-serine. The sites of enzymatic activity stain dark. The necrotic tubular epithelium in the subcortical zone contains phosphatase. Undamaged tubules in this location are devoid of cytoplasmic phosphatase. $\times 75$.

proximal convoluted tubules were damaged by *dl*-serine. In view of the irregularity of the staining reaction no conclusion could be drawn as to the nature of necrotic tubules that did not contain carmine. An additional source of error may be a nonspecific absorption of excreted carmine by necrotic cells.

The isolation of single units permitted exact localization of the tubular damage. In view of the extremely severe destruction in the nephrons, the damaged portions consisted of nothing but basement membranes containing cellular debris. They therefore fell apart easily. However, a sufficient number of nephrons from three kidneys could be isolated, and the localization of the lesion was made quite clear. The lesion was localized exclusively in the terminal portion of the proximal convolution (fig. 2). No evidence of necrosis was observed in the ascending limb, the distal convolution or elsewhere. There were, however, casts in distal convolutions and more in collecting tubules, but the epithelium in these regions was undamaged. The upper part of the proximal convolution was well preserved. In most of the preparations scattered fine dark round bodies were seen, which were interpreted by Dr. Oliver as droplets of some material, possibly of protein nature. These droplets were more concentrated near the glomerulus and gradually faded out, to end, as one's observation descended the proximal tubule, at about the beginning of its second third.

Relation of Amount of dl-Serine to the Extent of the Renal Damage.—As previously stated, 100 mg. of *dl*-serine produced severe renal damage in all rats to which it was given. In addition, 30 rats fed a complete diet were given 50 mg. of *dl*-serine dissolved in 3 cc. of water intraperitoneally. They showed changes comparable to those seen with the larger dose of 100 mg., though in some instances the necrosis was less extensive. In order to establish the smallest dose that would produce demonstrable renal lesions, 48 rats were divided into two groups; 21 rats fed a normal diet and 27 fed the synthetic diet were given a single intraperitoneal dose of *dl*-serine, varying between 10 and 30 mg., in 3 cc. of water. The lesions observed were graded as \pm , + and ++. Two pluses signified intense necrosis comparable to that seen with larger doses, although somewhat less extensive. Kidneys showing 1 plus lesions revealed areas of distinct necrosis. Here necrotic tubules in the inner portion of the cortex were frequently interspersed among undamaged tubules. Kidneys designated as having a plus-minus lesion showed only occasional frankly necrotic tubules. The damaged tubules showed more often fragmentation of the cytoplasm and pyknosis of nuclei.

As may be seen from table 1, a single dose of 10 mg. of *dl*-serine causes in most instances mild focal changes, while extensive necrosis was repeatedly observed following 20 to 30 mg. of *dl*-serine.

In 2 rats receiving intraperitoneally 500 mg. of *dl*-serine dissolved in 6 cc. of water the changes which occurred were essentially similar to those seen with 100 mg., although they appeared to be somewhat more extensive.

Relationship of Time to the Development of the Renal Lesion.—A rat killed incidentally ten hours after the administration of *dl*-serine

TABLE 1.—*Relation of the Amount of dl-Serine to the Extent of the Renal Changes*

Amount of dl-Serine, Mg.	Total Num- ber of Rats	Number Fed the Complete Diet Who Showed				Total Num- ber of Rats	Number Fed the Synthetic Diet Who Showed			
		No Lesions	± Lesions	+ Lesions	++ Lesions		No Lesions	± Lesions	+ Lesions	++ Lesions
10	1	1	0	0	0	6	1	5	0	0
15	4	2	1	1	0	9	0	1	6	2
20	4	1	2	0	1	8	1	1	0	6
25	6	0	1	1	4	2	0	1	1	0
30	6	0	3	3	0	4	0	0	1	3

revealed extensive necrosis indistinguishable from that seen after twenty-four hours. This observation prompted a study of the shortest time after which lesions of the kidneys would appear. For this purpose 45 rats were given the standard dose of 100 mg. of *dl*-serine; 20 animals received the drug intravenously, and 27, intraperitoneally. As

TABLE 2.—*Relationship of Time to the Development of the Renal Lesion*

Time Elapsing After Administration of dl-Serine Before Animals Were Killed, Minutes	Total Number of Rats	Number Showing Demonstrable Lesions
10.....	1 (1)*	0 (0)*
15.....	2 (2)	0 (0)
20.....	2 (2)	0 (0)
25.....	1 (1)	0 (0)
30.....	4 (4)	2 (2)
60.....	2 (1)	2 (1)
75.....	3 (2)	3 (2)
90.....	3 (1)	3 (1)
100.....	1 (1)	1 (1)
105.....	2 (1)	2 (1)
120.....	6 (1)	6 (1)
150.....	2	2
180.....	8 (1)	8 (1)
240.....	4 (1)	4 (1)
300.....	4 (1)	4 (1)
360.....	2	2

* The figures in parentheses are the numbers of rats given the *dl*-serine by intravenous injection.

may be seen in table 2, no definite lesions were observed earlier than thirty minutes after the intravenous injection. Among 4 rats 1 showed slight and 1 fairly marked evidence of renal injury thirty minutes after intravenous administration of *dl*-serine (fig. 3). All rats killed after seventy-five to three hundred and sixty minutes showed renal damage. In the early stages the damaged cells revealed often marked eosinophilia in their cytoplasm, while the nuclei appeared to be less intensely stained than those in uninvolved tubules. The cytoplasm

at the same time appeared shrunken, so that the height of the cell was less than that of cells lining uninvolved tubules. In many of the damaged proximal convoluted tubules, the brush border was separated from the eosinophilic cytoplasm and stood out clearly. In some of the tubules the cytoplasm showed considerable fragmentation, while the nuclei were still intact. Often the luminal portion of the cytoplasm seemed to break off, forming sometimes irregularly shaped eosinophilic clumps and at other times rounded, more basophilic structures. Lesions of this kind were mainly seen in the kidneys of rats killed up to three hours after injection, while in the following hours the damaged tubules showed more and more the picture of diffuse necrosis with complete loss of all structures of the cytoplasm and disappearance of the nuclei. However, distinct necrosis was seen in some of the tubules as early as thirty to sixty minutes after administration of *dl*-serine. The change was focal in the early stages and became more diffuse later on. Litter mates which received intraperitoneal and intravenous injections simultaneously and were killed after intervals varying from seventy-five to three hundred minutes revealed no significant differences in the extent and severity of the renal damage.

Influence of Weight.—Five adult male rats and 5 weanling rats were used. Three rats weighing between 170 and 190 Gm. received each 100 mg. of *dl*-serine subcutaneously. Two rats weighing 185 Gm. and 280 Gm., respectively, received 100 mg. of *dl*-serine for each 100 Gm. of body weight. In all 5 rats extensive necrosis was observed. No significant difference was noticed between those given the smaller and those given the larger dose. Of the 5 weanling male rats weighing 35 to 40 Gm., 2 were given 25 mg. and 3 were given 50 mg. of *dl*-serine. In 4 of these animals the extent of renal damage was similar to that seen in the standard 100 Gm. rat. The necrotic changes, although present, were slight in only 1 rat.

Influence of Sex.—Of 11 female rats weighing approximately 100 Gm., 5 were given 100, 2 were given 50 and 4 were given 30 Gm. of *dl*-serine intraperitoneally. Of the 5 rats receiving 100 mg., 4 showed renal damage fully comparable to that seen in male rats, while in 1 no lesions developed. In the 2 animals receiving 50 mg., extensive renal necrosis was observed. Of the 4 rats receiving 30 mg., 2 showed considerable, 1 slight and 1 no necrosis.

In addition, 6 adult female rats were given the *dl*-serine. Three weighing between 195 and 235 Gm. received 100 mg. by intraperitoneal injection. Of these, 2 showed considerable necrosis, while in 1 only slight degenerative changes and no renal necrosis was found. Three other rats weighing from 205 to 250 Gm. were given 100 mg. *dl*-serine per hundred grams of body weight. Of these, 1 showed marked and 1 moderate renal necrosis, and in 1 the kidneys remained unchanged.

Phosphatase Activity.—In the rat's kidney alkaline phosphatase activity is found predominantly in the cortical portion.¹⁵ Cytoplasmic activity is seen mostly in the proximal convoluted tubules, while the tubular structures in the medulla show phosphatase in the nuclei only. Phosphatase activity is seen also in the glomeruli,^{15d} in the adventitia of larger arteries and veins and in the capillaries of the medulla.

In 8 animals phosphatase activity was examined twenty-four hours after administration of *dl*-serine. The necrotic tubules still contained the enzyme. They were uniformly and deeply stained. A similar reaction was given by desquamated cell masses. Some of the casts also revealed enzymatic activity. Undamaged tubules devoid of cytoplasmic phosphatase in the subcortical zone were apparently more numerous than in sections of normal controls (fig. 4). No changes in phosphatase activity were noticed in any of the proximal convoluted tubules situated in the more peripheral portions of the renal cortex.

COMMENT

The results reported here fully confirm the findings of Morehead and co-workers¹⁶ concerning the nephrotoxic action of *dl*-serine. When a dose of 100 mg. was given to male rats weighing 100 Gm. either by stomach tube or by intraperitoneal injection, all the animals showed typical renal necrosis at the corticomedullary junction within twenty-four hours. The damage was somewhat more severe in animals fed for seven days on a synthetic diet deficient in the B vitamins and poor in protein. Changes of similar degree and severity were seen in most rats given half the dose with only few exceptions, in which necrosis, although present, was less extensive. Five hundred milligrams *dl*-serine given in a single dose did not essentially influence the extent of the renal damage.

An attempt was made to establish the minimum amount of the amino acid capable of exerting a nephrotoxic action. As little as 10 mg. of *dl*-serine given to male rats weighing 100 Gm. and fed the synthetic diet led to focal degenerative changes, while 20 to 30 mg. produced extensive necrosis in a number of animals. Since only the unnatural *d*-isomer in the racemic mixture exerts apparently a nephrotoxic action,⁶ the minimal kidney-damaging dose may be as low as 5 mg. In this connection the observations of Fishman and Artom¹⁷

15. (a) Gomori, G.: *J. Cell. & Comp. Physiol.* **17**:71, 1941. (b) Bourne, G.: *Quart. J. Exper. Physiol.* **32**:1, 1943. (c) Menten, M. L.; Junge, J., and Green, M. H.: *Proc. Soc. Exper. Biol. & Med.* **57**:82, 1944. (d) Wachstein, M.: *Arch. Path.* **38**:297, 1944; (c) *J. Exper. Med.* **84**:25, 1946.

16. Morehead, Fishman and Artom (footnotes 3 and 5).

17. Fishman, W. H., and Artom, C.: *Proc. Soc. Exper. Biol. & Med.* **60**:288, 1945.

are of interest, who found that 6 to 13 per cent of a 100 mg. dose of *dl*-serine is excreted unchanged in the urine.

The rapidity with which the anatomically demonstrable lesion may occur after administration of *dl*-serine merits some discussion. Evidence of renal damage was visible after only thirty minutes in a few instances, and degenerative changes were regularly noticed within sixty to seventy-five minutes. These degenerative changes increased in severity in the course of the next hours, and after four to five hours frank necrosis was quite extensive in most of the examined kidneys.

Only few reports could be found mentioning the earliest microscopic evidence following the administration of nephrotoxic substances. Kosugi¹⁸ found evidence of tubular damage not earlier than six hours after the administration of mercury bichloride in rats. Ginzler,¹⁹ however, stated that degenerative lesions could be seen in rabbits four hours after intravenous injection of mercury bichloride. After chromium had been administered, no microscopic lesions were seen within ten hours.²⁰ Hydropic changes of the epithelium of the convoluted tubules in rabbits occurred two and a half to five and a half hours after the injection of tartaric acid.²¹ Within from one to two hours²² after sodium oxalate had been administered intravenously to rabbits, necrosis was found in some of the convoluted tubules and even still earlier²³ with larger doses. Mitochondrial changes were seen in rabbits twenty minutes after the intravenous injection of uranium nitrate, while the first microscopic evidence of renal damage was noticed in sections stained with hemalum and eosin within two hours.²³

More recently Gilman and co-workers²⁴ found sodium tetrathionate to be extremely nephrotoxic. Advanced renal necrosis was observed in dogs within thirty minutes after intravenous injection of this substance.

The nephrotoxic action of *dl*-serine is not restricted to rats weighing 100 Gm. but can be produced in weanling as well as in adult animals. Fishman and Artom¹ noticed a considerable resistance of female rats against the toxic effects of prolonged administration of *dl*-serine. However, renal lesions occur also in female rats, although not with the same regularity as in males. The fact that sex differences exist in

18. Kosugi, T.: Beitr. z. path. Anat. u. z. allg. Path. **77**:1, 1927.

19. Ginzler, A. M.: Personal communication to the author.

20. Eichler, O.: Handbuch der experimentellen Pharmakologie, Berlin, Julius Springer, 1924, vol. 3, pt. 3, p. 1526.

21. Potter, A. C., and Bell, E. T.: Am. J. M. Sc. **149**:236, 1915.

22. Dunn, J. S.; Haworth, A., and Jones, N. A.: J. Path. & Bact. **27**:299, 1924.

23. Gough, J.: J. Path. & Bact. **34**:423, 1931.

24. Gilman, A.; Philips, F. S.; Koelle, E. S.; Allen, R. P., and John, E. St.: Am. J. Physiol. **147**:115, 1946.

the kidney's reactivity to nephrotoxic influences has been previously established. The typical hemorrhagic necrosis which occurs in young rats fed choline-deficient diets is more common in males than in females.²⁵ After oral administration of chloroform, Eschenbrenner²⁶ found renal necrosis in male but not in female mice. Male rats showed a higher incidence of necrotizing nephrosis when exposed to repeated inhalation of carbon tetrachloride vapors than female animals.²⁷

The peculiar location of the necrotizing lesion of the kidney suggested that a detailed analysis of the involved tubules be made. The general localization of the renal damage in the subcortical zone made obvious that the distal portion of the proximal convoluted tubules, the upper portion of the ascending loops of Henle and portions of the collecting tubules could be involved. Attempts to utilize the vital staining technic of Suzuki did not lead to unequivocal results. The most satisfactory technic for the localization of functional and pathologic processes in certain parts of the renal tubules, consists in the isolation of single renal units, as pointed out by Oliver.¹⁰ With the help of this technic, Edwards²⁸ was able to demonstrate that mercury bichloride damages predominantly the second and third quarters of the proximal convoluted tubules, while the most distal portions were usually spared.

In isolated and appropriately stained nephrons the *dl*-serine damage was found to be localized in the distal portions of the proximal convoluted tubules, whereas all other structures were spared. The results of this technic is most revealing in view of the extensive involvement of the subcortical zone, which would lead one to believe that not only the proximal convoluted tubules but also the ascending limbs of Henle are involved in the morbid process. Morehead and co-workers³ stated that necrosis was most marked in the innermost portion of the cortex but sometimes extended toward the periphery to involve the descending portions of the proximal convoluted tubules, obviously assuming that Henle's loops were predominantly injured. The findings reported here are in line with the well known susceptibility of the proximal convoluted tubules to various renal poisons.

In view of the fact that only the terminal portion of the proximal convoluted tubule is damaged, *dl*-serine should prove a valuable tool for producing a strictly localized renal lesion. In contrast to mercury and uranium, it does not involve other segments of the nephron regardless of the amount given. In addition, the lesions can invariably be produced with adequate doses in male animals.

25. Griffith, W. H.: *J. Nutrition* **19**:437, 1940.

26. Eschenbrenner, A. B.: *J. Nat. Cancer Inst.* **5**:251, 1945.

27. György, P.; Seifter, J.; Tomarelli, R. M., and Goldblatt, H.: *J. Exper. Med.* **83**:449, 1946.

28. Edwards, J. G.: *Am. J. Path.* **18**:1011, 1942.

In the kidney of the rat cytoplasmic phosphatase activity is marked in the cortex and in the subcortical zone. In the latter, most of the tubules show enzymatic activity, while only occasionally some are devoid of it. It still is an unsettled question whether the ascending loops of Henle participate in this reaction, as stated by Menten and co-workers.^{15c} The experience with *dl*-serine clearly demonstrated that in tissue sections most of the tubules located in the subcortical zone are proximal convolutions. Since no cytoplasmic phosphatase activity is seen in the ascending limbs, located in the medulla, it seems reasonable to assume that these structures in the rat kidney do not show enzymatic activity. This circumstantial evidence is strengthened by the fact that after administration of *dl*-serine only necrotic tubules in the subcortical zone revealed phosphatase, while the cytoplasm of undamaged structures remained unstained. It must, however, be conceded that the final solution of this question will have to wait until isolated renal units can be examined with the phosphatase stain.

Cells made necrotic by the action of *dl*-serine contained the enzyme in the diffuse form. Similarly the necrotic cells in the acute phase of poisoning with uranium,²⁹ mercury bichloride ^{29a,b,d} and potassium dichromate ^{29a,b} still revealed enzymatic activity.

SUMMARY

The findings of Fishman, Artom and Morehead concerning the nephrotoxic action of *dl*-serine in rats were fully confirmed. Not only male animals weighing 100 Gm. but also weanling and adult rats are susceptible. In female rats *dl*-serine exerts likewise a nephrotoxic action, although less regularly.

The minimal injurious dose for male rats weighing 100 Gm. and fed a synthetic diet poor in the B vitamins and deficient in protein was found to be 10 mg., while 20 to 30 mg. led in most animals to extensive necrosis. Distinct renal lesions were observed in some instances as early as thirty minutes after intravenous injection of *dl*-serine.

By vital staining with a lithium-carmin solution and by using the maceration and dissection method of Oliver, the damage of the kidney was localized in the terminal portions of the proximal convoluted tubules. After the introduction of *dl*-serine, phosphatase activity was still found twenty-four hours later in the cells of necrotic tubules. The restriction of the damage to the distal portion of the convoluted tubules and its regular occurrence suggest that *dl*-serine would be a valuable tool for the production of localized renal injury.

29. (a) Hepler, O. E.; Simmonds, J. P., and Gurley, H.: Proc. Soc. Exper. Biol. & Med. **44**:221, 1940. (b) Hepler, O. E.; Gurley, H., and Simmonds, J. P.: Arch. Path. **39**:133, 1945. (c) Breedis, C.; Flory, C. M., and Furth, J.: *ibid.* **36**:402, 1943. (d) Wachstein,^{15c}

NEPHROTOXIC ACTION OF *DL*-SERINE IN THE RAT

II. The Protective Action of Various Amino Acids and Some Other Compounds

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IN THE preceding communication¹ the observations of Fishman, Artom and Morehead² concerning the injurious action of *dl*-serine on the rat kidney were fully confirmed. In amplification of these experiments it was found that not only male rats weighing 100 Gm. but weanling as well as adult animals are susceptible. Kidneys of female rats are likewise damaged, although less frequently. As little as 10 mg. may exert a nephrotoxic action in male rats weighing 100 Gm. Microscopically demonstrable renal damage may appear as early as thirty minutes after intravenous administration. The necrosis is limited to the terminal portion of the proximal convoluted tubules.

In agreement with Morehead, Fishman and Artom,^{2b} renal changes were found to be more extensive on a synthetic diet poor in protein and deficient in B vitamins. These investigators found that addition of pure B vitamins to this diet reduced considerably the mortality and the severity of the clinical symptoms but did not influence the extent of the renal lesions.³ Addition of choline hydrochloride, glycine and *l*-cystine to the diet had likewise no beneficial influence on the renal damage.

The experiments reported here were started in view of the evidence brought forward recently that serine and methionine react in the animal body to form cystine.⁴ It seemed of interest, therefore, to investigate whether methionine could influence the injurious action of *dl*-serine. Furthermore, it is known that renal lesions occur in weanling rats on

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1. Wachstein, M.: Arch. Path., this issue, p. 503.

2. (a) Fishman, W. H., and Artom, C.: J. Biol. Chem. **145**:345, 1942.

(b) Morehead, R. W.; Fishman, W. H., and Artom, C.: Am. J. Path. **21**:803, 1945.

3. Fishman, W. H., and Artom, C.: Proc. Soc. Exper. Biol. & Med. **57**:241, 1944. Morehead, R. W.; Fishman, W. H., and Artom, C.: Am. J. Path. **22**:385, 1946.

4. (a) Stetten, D.: J. Biol. Chem. **144**:501, 1942. (b) Binkley, F., and du Vigneaud, V.: *ibid.* **144**:507, 1942. (c) Du Vigneaud, V.; Kilmer, G. W.; Rachele, J. R., and Cohn, M.: *ibid.* **155**:645, 1944.

various choline-free diets.⁵ The renal changes are aggravated by simultaneous feeding of cystine and prevented by methionine.⁶ Although the renal necrosis occurring in this condition is localized predominantly in the outer portion of the cortex and is of a hemorrhagic type, it seemed possible that the renal damage observed after *dl*-serine was due to the transformation of this substance into cystine.

In view of the beneficial action which *dl*-methionine was found to exert, a number of substances containing sulfhydryl groups, as well as other compounds, were tested for their protective influence.

MATERIAL AND METHODS

Litters containing 5 to 8 male albino rats of the Wistar strain, weighing about 100 Gm., were used for all experiments. In each litter 1 or 2 animals served as controls. In testing the protective action of *dl*-methionine, the animals were fed either "Rockland rat diet complete" (Arcady Farms, Milling Co., Chicago) or, for a period of seven days, a protein-deficient, vitamin B complex-free diet of a composition similar to that of the diet 1 used by Fishman and Artom.^{2a} This diet consisted of vitamin test casein (General Biochemicals) 10 per cent, dextrin 39 per cent, sucrose 37 per cent, hydrogenated cottonseed oil ("crisco") 5 per cent, cod liver oil 5 per cent and salt mixture no. 2 U. S. P. XII 4 per cent. *dl*-Methionine was injected subcutaneously in doses of 100 mg. dissolved in 3 cc. of distilled water.

Animals protected with six doses of methionine received the amino acid morning, noon and evening on two consecutive days. On the second day the first dose of *dl*-methionine was given one hour before 100 mg. of *dl*-serine was administered by stomach tube. The next dose was given three to four hours later, and the last dose was given in the evening. Animals protected by five doses received the first injection at noon of the first day. Those protected with four doses of *dl*-methionine received it on the evening of the first day, while those receiving three doses of methionine were given it on the same day that *dl*-serine was administered. To those animals receiving two injections the *dl*-methionine was given one hour before and three to four hours after the test dose of *dl*-serine. Rats serving as controls received only 100 mg. of *dl*-serine by stomach tube and were treated with an equivalent volume of distilled water, injected subcutaneously.

Since no definite difference was seen in the methionine protection experiments between animals fed the complete and those fed the synthetic diet, the complete diet was used in the experiments with other compounds. *l* (+) Cysteine hydrochloride, sodium thioglycollate, glutathione, 2,3-dithiopropanol (BAL), *dl*-alpha-alanine, glycine, *dl*-threonine, *l* (+) glutamic acid, *l* (+) histidine monohydrochloride, *l* (+) arginine monohydrochloride, *dl*-valine, glycollic acid, butyric acid, pyruvic acid, sodium lactate, *d*-glucose, sodium chloride and sodium acetate were tested for their protective action.

In view of the fact elicited in the course of this work¹ that well within thirty minutes after the administration of *dl*-serine renal changes were microscopically demonstrable, the other tested compounds were given by three subcuta-

5. Griffith, W. H., and Wade, N. J.: J. Biol. Chem. **131**:567, 1939. György, P., and Goldblatt, H.: J. Exper. Med. **72**:1, 1940. Engel, R. W., and Salmon, W. D.: J. Nutrition **22**:109, 1941. Christensen, K.: Arch. Path. **34**:633, 1942. Wachstein, M.: *ibid.* **38**:297, 1944.

6. Griffith, W. H., and Wade, N. J.: J. Biol. Chem. **132**:627 and 639, 1940. Griffith, W. H.: J. Nutrition **21**:291, 1941.

neous injections, fifteen minutes before and fifteen and forty-five minutes after the intraperitoneal injection of a test dose of 50 mg. of *dl*-serine. The animals were likewise killed twenty-four hours later. *dl*-Serine was dissolved in 3 cc. of distilled water, while most of the tested substances were dissolved in 2 cc. of distilled water and the hydrogen ion concentration adjusted approximately to p_H 7.2. In most instances each injection contained 100 mg. of the tested substance. As in the previous experiments, at least 1 litter mate served as a control, receiving the same amount of *dl*-serine and injections of distilled water in equivalent amounts. Certain exceptions to this schedule were made, which will be referred to later.

RESULTS

Effect of dl-Serine.—The renal changes occurring in rats fed the complete and those fed the synthetic diet after the administration of *dl*-serine were described in the preceding paper.¹ They occurred regularly after the administration of 50 to 100 mg. of *dl*-serine. On gross

TABLE 1.—Summary of the Results of Experiments to Determine the Extent to Which Various Doses of *dl*-Methionine Would Protect Rats Fed a Complete Diet Against Renal Injury Caused by Intragastric Administration of 100 Mg. of *dl*-Serine.

Number of Subcutaneous Injections of <i>dl</i> -Methionine (100 Mg. in 3 Cc. of Water)	Total Number of Rats	Number of Rats Presenting		
		Maximal Lesions	Moderate Lesions	Slight or No Lesions
6.....	18	18		
5.....	3	3
4.....	2	1	..	1
3.....	3	3
2.....	6*	..	2	4*
1.....	3	..	1	2
0.....	3	..	2	1

* Three rats received the three injections of *dl*-methionine within one hour.

examination a small, irregularly shaped strip of grayish discoloration which was seen at the innermost portion of the cortex indicated the necrotic area. Microscopically, the changes found were similar to those described by Morehead, Fishman and Artom.^{2b} Acute renal necrosis occurred at the junction of the cortex and the medulla twenty-four hours after the administration of *dl*-serine. The epithelium of many tubules in this area was almost completely necrotic (figs. 1 and 3).

The changes observed varied somewhat in severity in different fields. Casts composed of eosinophilic granular material were frequent, located at times in the convoluted, but mostly in the collecting tubules. Not all the tubules at the corticomedullary junction were necrotic. Even where the damage was most extensive, occasional tubules were spared. In animals kept on the synthetic diet the renal damage induced by *dl*-serine was more extensive. In some fields the necrosis extended into the more outlying portions of the cortex. The glomeruli were not affected.

Effect of dl-Methionine on the Nephrotoxic Action of dl-Serine.—The influence of *dl*-methionine on the renal damage caused by *dl*-serine was examined in 42 rats. The results may be seen in tables 1 and 2.

"No lesions" and "slight lesions" signify complete absence of any damage (fig. 2) and the occasional degenerative changes noticed in some of the tubules in the inner portion of the cortex, respectively. In kidneys designated as showing moderate renal lesions, focal necrosis of cortical tubules was present. This necrosis was much less conspicuous than that seen in litter mates not protected by *dl*-methionine. In only 2 of all the animals was no protection found. The protective effect of *dl*-methionine was apparent in the animals on the protein-deficient diet as well as in those on the normal diet.

Effect of Some Other Amino Acids and Various Additional Compounds on the Nephrotoxic Action of dl-Serine.—The results of experi-

TABLE 2.—*Summary of Results of Experiments to Determine the Extent to Which Various Doses of dl-Methionine Would Protect Rats Which for Seven Days Had Been Restricted to a Protein-Deficient, B Vitamin-Free Diet Against Renal Injury Caused by Intragastric Administration of 100 Mg. of dl-Serine*

Number of Subcutaneous Injections of dl-Methionine (100 Mg. in 3 Cc. of Water)	Total Number of Rats	Number of Rats Presenting		
		Maximal Lesions	Moderate Lesions	Slight or No Lesions
6.....	13	13		
5.....	4	4
4.....	3	3
3.....	3	3
2.....	4	4
1.....	3	3
	5	1	2	2

ments on the protective action of some other amino acids and various additional substances against the nephrotoxic action of *dl*-serine are listed in table 3.

1. *l* (+) Cysteine hydrochloride. Of 10 animals given 100 mg. of *l* (+) cysteine hydrochloride three times, only 4 survived for a period of twenty-four hours. All of them showed the same amount of renal necrosis as the controls. Of 11 rats given 50 mg. of *l* (+) cysteine hydrochloride three times, 8 survived; 6 showed no protection; in 2 rats renal necrosis, although present, was less severe.

In order to test the possible damaging influence of cystine and cysteine, 8 normal rats restricted for seven days to the protein-deficient diet were given 45 mg. of *l* (—) cystine, and a second group of 8 normal rats received 90 mg. of *l* (—) cystine suspended in 3 cc. of distilled water daily by stomach tube for a period of seven days. These animals were killed on consecutive days. With exception of congestion of blood vessels in some of the kidneys, no lesions were found comparable to those occurring after administration of *dl*-serine.

Two normal animals fed the protein-deficient diet received two, and 3 normal animals received three administrations of 130 mg. of *l* (+)

cysteine hydrochloride in 3 cc. of water by stomach tube on consecutive days. In addition, 4 rats were given 150 mg. by subcutaneous injection. Necrotic changes were not found in the kidneys of any of these animals.

2. Sodium thioglycollate. Of 27 rats given 40 to 90 mg. of sodium thioglycollate by two subcutaneous injections (divided doses) administered within one hour, 15 survived twenty-four hours. The first dose, varying between 20 and 50 mg., was followed fifteen to thirty minutes

TABLE 3.—Summary of Results of Experiments to Determine Whether Various Other Amino Acids and Some Other Compounds Would Protect Rats Fed a Complete Diet Against Renal Injury Caused by Intraperitoneal Injection of 50 Mg. of dl-Serine

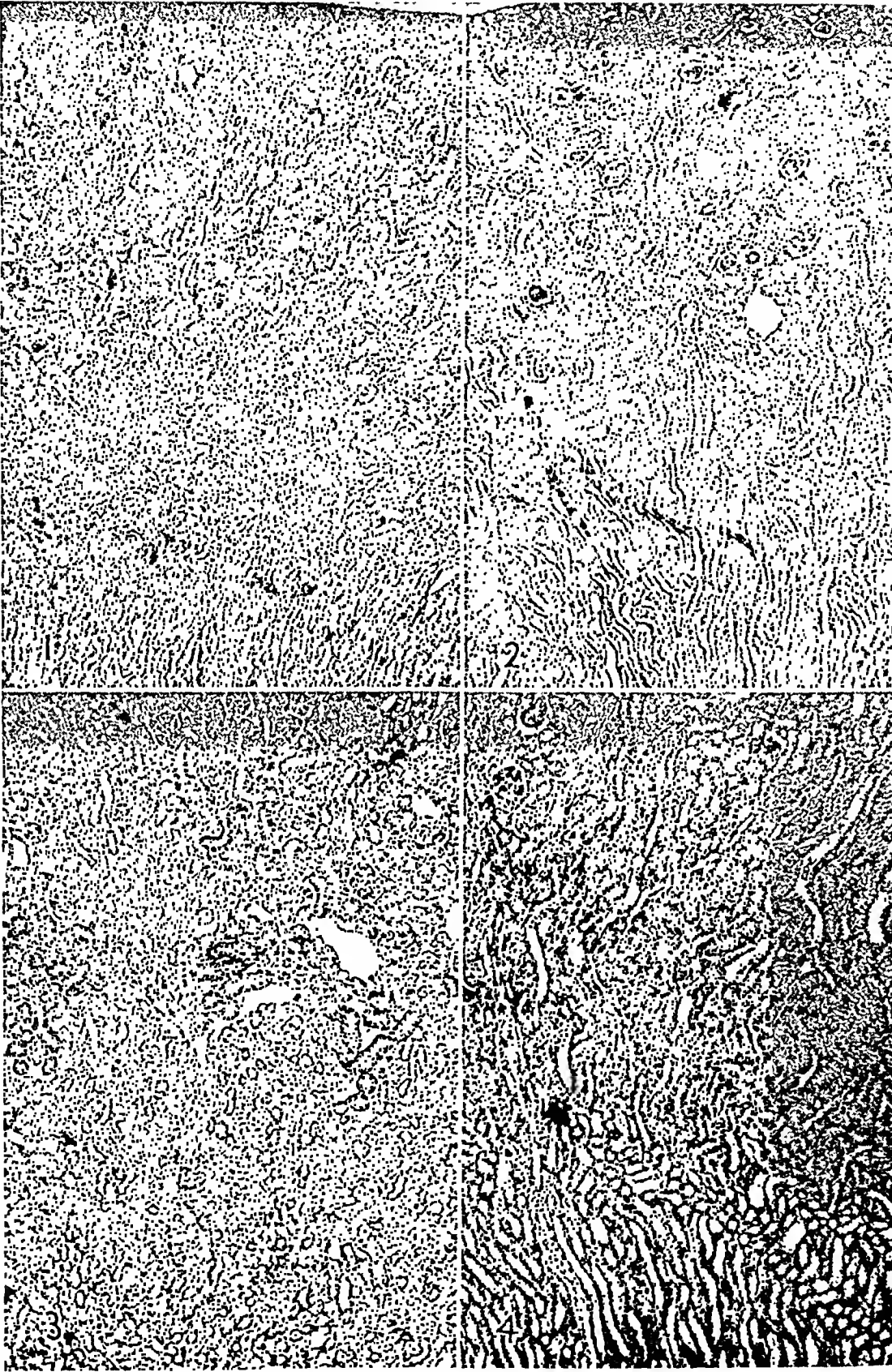
Substance Tested	Amount, Mg.	Mode of Injection	Total Number of Rats	Number of Rats Showing		
				Maxi- mal Lesions	Mod- erate Lesions	Slight or No Lesions
1 (+) Cysteine hydrochloride	300	Subcutaneous	30	30		
1 (+) Cysteine hydrochloride	150	Subcutaneous	4	4		
Sodium thioglycollate.....	40	Subcutaneous	8	6	2	
Sodium thioglycollate.....	45	Subcutaneous	2	2		
Sodium thioglycollate.....	50	Subcutaneous	4	4		
Sodium thioglycollate.....	50	Subcutaneous	1	1		
Sodium thioglycollate.....	60	Subcutaneous	1	1		
Sodium thioglycollate.....	80	Subcutaneous	3	..	1	2
Sodium thioglycollate.....	90	Subcutaneous	4	3	1	
Glutathione.....	300	Subcutaneous	12	1	..	11
2,3-Dithiopropanol (BAL)....	20	Intramuscular	1	1		
2,3-Dithiopropanol (BAL)....	10	Intramuscular	2	2		
2,3-Dithiopropanol (BAL)....	3	Intramuscular	3	3		
dl-Alpha-alanine.....	300	Subcutaneous	10	1	..	10
Glycine.....	300	Subcutaneous	10	1	..	9
dl-Threonine.....	300	Subcutaneous	9	9
1 (+) Glutamic acid.....	300	Subcutaneous	3	1	1	1
1 (+) Glutamic acid.....	150	Subcutaneous	5	5		
1 (+) Histidine monohydro- chloride.....	360	Subcutaneous	8	1	4	3
1 (+) Arginine monohydro- chloride.....	360	Subcutaneous	9	3	4	2
dl-Valine.....	300	Subcutaneous	8	2	5	1
Glycollic acid.....	180	Subcutaneous	12	..	1	11
Butyric acid.....	300	Subcutaneous	7	..	3	4
Pyruvic acid.....	300	Subcutaneous	7	..	2	5
Sodium lactate.....	300	Subcutaneous	7	1	6	
d-Glucose.....	300	Subcutaneous	7	7		
Sodium chloride.....	300	Subcutaneous	3	3		
Sodium acetate.....	300	Subcutaneous	5	5		

later by the test dose of dl-serine. The second dose of sodium thioglycollate in the amount of 20 to 40 mg. was given fifteen to fifty minutes later.

As may be seen from table 3, little protection was afforded by sodium thioglycollate. Only large doses, which were not tolerated by other rats, influenced the renal damage in some instances.

3. Glutathione. Of 12 animals given 100 mg. of glutathione three times, complete protection was afforded 11. Only in 1 animal was no beneficial effect seen.

4. 2, 3-Dithiopropanol (BAL). BAL in peanut oil containing 20 per cent benzyl benzoate (Hynson, Westcott & Dunning, Inc., Baltimore)



Figures 1 to 4.

(See legend on opposite page)

was used. Three animals were given one intramuscular injection of 0.1 cc. and 3 one intramuscular injection of 0.2 cc. containing 10 and 20 mg. of BAL, respectively. Thirty minutes later the test dose of *dl*-serine was administered. Two rats treated with the smaller dose, and 1 with the larger, survived for twenty-four hours. In addition, 3 other rats were given three injections of 1 mg. of BAL, the first dose being administered thirty minutes before the *dl*-serine, the second and the third dose fifteen and thirty minutes after. In none of these animals was any influence on the renal damage caused by *dl*-serine noticed.

5. *dl*-Alpha-alanine. Ten animals were given 100 mg. of *dl*-alpha-alanine three times. The kidneys of all of them were completely protected.

6. Glycine. Ten rats were given 100 mg. of glycine in three equal doses. In 9 of them complete protection occurred, while in 1 animal the necrotic lesions of the kidneys were not influenced.

In order that the influence of glycine itself on the kidneys might be examined, 4 normal rats were given 300 mg. of glycine dissolved in 3 cc. of distilled water. No appreciable renal lesions were found.

7. *dl*-Threonine. Nine animals were given 100 mg. of *dl*-threonine three times. All of them showed complete protection (fig. 4).

In view of the near chemical relationship of threonine and serine, 2 normal rats were given 100 and 3 were given 500 mg. of the substance intraperitoneally. No renal changes were seen.

8. *l* (+) Glutamic acid. Of 7 animals given 100 mg. of *l* (+) glutamic acid three times, only 3 survived. Six additional rats were given 50 mg. three times, of which 5 survived twenty-four hours. No influence was seen from the smaller dose, while the larger afforded some protection.

EXPLANATION OF FIGURES 1 TO 4

Fig. 1.—Section of the kidney of a male rat fed the synthetic diet and killed twenty-four hours after the administration of 100 mg. of *dl*-serine by stomach tube. There is extensive necrosis of tubular epithelium in the subcortical zone. Hematoxylin and eosin; $\times 37.5$.

Fig. 2.—Section of the kidney of a litter mate of the rat furnishing the section shown in figure 1. This litter mate was protected by three subcutaneous injections of 100 mg. of *dl*-methionine and given the same amount of *dl*-serine. Necrotizing nephrosis is completely absent. Hematoxylin and eosin; $\times 37.5$.

Fig. 3.—Section of the kidney of a male rat fed the complete diet and killed twenty-four hours after an intraperitoneal injection of 50 mg. of *dl*-serine. There is considerable necrosis of the tubular epithelium in the subcortical zone. Hematoxylin and eosin; $\times 55$.

Fig. 4.—Section of the kidney of a litter mate of the rat furnishing the section shown in figure 3. This litter mate was protected by three subcutaneous injections of 100 mg. of *dl*-threonine. There is complete absence of renal necrosis. Hematoxylin and eosin; $\times 55$.

9. *l* (+) Histidine monohydrochloride. Of 8 animals protected with 120 mg. of *l* (+) histidine monohydrochloride three times, 3 showed no renal lesions, while in 4 necrosis, although present, was less marked than in the unprotected controls. In 1 animal no protection was seen.

10. *l* (+) Arginine monohydrochloride. Of 9 animals given 120 mg. of *l* (+) arginine monohydrochloride three times, 3 showed severe renal lesions, while moderate protection was found in 4 and complete in 2.

11. *dl*-Valine. Eight rats received 100 mg. of *dl*-valine three times. No protection was seen in 2, while protection was complete in 1 and moderate in 5.

12. Glycollic acid. Of 12 rats given 60 mg. of glycollic acid three times, 11 showed complete protection, while in 1 the protection was less complete.

13. Butyric acid. The hydrogen ion concentration of the butyric acid could be adjusted to only about p_H 4.2. Of 7 rats given 100 mg. three times, no renal lesions were found in 4 and moderate lesions were found in 3.

14. Pyruvic acid. Of 7 rats given three equal doses of 100 mg. of pyruvic acid, 5 showed complete absence of the typical lesions of the kidneys, and 2 showed moderate lesions. However, there was a varying amount of hydropic degeneration in the proximal convoluted tubules located in the outer portion of the cortex. In 3 normal rats given 300 mg. of pyruvic acid dissolved in 6 cc. of distilled water similarly, some hydropic degeneration in the proximal convoluted tubules was seen. Necrotic changes were completely absent.

15. Sodium lactate. Of 7 animals given three injections of 100 mg. of sodium lactate, no protection was seen in 1, while moderate protection was found in 6.

16. *d*-Glucose. Seven animals received three doses of 100 mg. of *d*-glucose. No influence on the extent and severity of the renal lesions was seen.

17. Sodium chloride. In 3 rats given 100 mg. of sodium chloride in three doses, the extent of the renal lesions was not influenced.

18. Sodium acetate. Five animals given 100 mg. of anhydrous sodium acetate in three equal doses revealed renal changes that were not different from those of the controls.

COMMENT

A considerable protection of methionine as well as of other substances against the nephrotoxic action of *dl*-serine could be demonstrated in these experiments.

A protective action of methionine against the chloroform injury of the liver in protein-depleted dogs⁷ and normal dogs⁸ has been previously established. Methionine was shown to exert a beneficial effect on the hepatic injury in protein-depleted dogs given oxophenarsine hydrochloride ("mapharsen").⁹ It also protected mice fed a complete¹⁰ and rats fed a protein-deficient diet¹¹ against dichloroethane poisoning. Methionine failed to exert a protective action in rats and dogs on a complete diet poisoned with carbon tetrachloride.¹² György and co-workers¹³ found that methionine and methionine-containing proteins protected against renal injury more consistently than against hepatic damage in rats exposed to the inhalation of carbon tetrachloride. *dl*-Methionine prevents to a considerable extent the severe degenerative changes induced in the liver and the kidneys of young rats by pyridine.¹⁴

Of other substances containing a sulfhydryl group tested, little effect from sodium thioglycollate and from cysteine and none from BAL was seen. BAL given in appropriate amounts prevents completely the renal injury occurring in mercury bichloride poisoning¹⁵ and following the administration of sodium tetrathionate.¹⁶ It can therefore be reasonably assumed that the serine damage is not due to interference with SH groups and that the protective action of methionine and glutathione, the other effective sulfhydryl-bearing substances tested, is not due to their SH group.

Glutathione is a tripeptide of glutamic acid, cysteine and glycine. Of these three substances, cysteine, as already stated, had little protective action. *l* (+) Glutamic acid had only moderate protective influence.

7. Miller, L. I.; Ross, J. F., and Whipple, G. H.: *Am. J. M. Sc.* **200**:739, 1940. Miller, L. I., and Whipple, G. H.: *J. Exper. Med.* **76**:421, 1942.

8. Brunschwig, S.; Nichols, S.; Bigelow, R. R., and Mills, J.: *Arch. Path.* **40**:81, 1945.

9. Goodell, J. P. B.; Hanson, P. C., and Hawkins, W. B.: *J. Exper. Med.* **79**:625, 1944.

10. Heppel, L. A.; Neal, P. A.; Perrin, L. T.; Endicott, R. M., and Porterfield, V. A.: *J. Pharmacol. & Exper. Therap.* **84**:53, 1945.

11. Heppel, L. A.; Neal, P. A.; Daft, F. S.; Endicott, K. M.; Orr, M. L., and Porterfield, V. A.: *J. Indust. Hyg. & Toxicol.* **27**:15, 1945.

12. Brunschwig, A.; Johnson, C., and Nichols, S.: *Proc. Soc. Exper. Biol. & Med.* **60**:388, 1945. Shaffer, C. B.; Carpenter, C. P., and Moses, C.: *J. Indust. Hyg. & Toxicol.* **28**:87, 1946. Drill, V. A., and Loomis, T. A.: *Science* **103**:199, 1946.

13. György, P.; Seifter, J.; Tomarelli, R. M., and Goldblatt, H.: *J. Exper. Med.* **83**:449, 1946.

14. Baxter, H.: *J. Clin. Investigation* **25**:908, 1946.

15. Ginzler, A. M.: *Federation Proc.* **5**:221, 1946. Gilman, A.; Allen, R. P.; Philips, F. S., and John, E. St.: *J. Clin. Investigation* **25**:549, 1946.

16. Gilman, A.; Philips, F. S.; Koelle, E. S.; Allen, R. P., and John, E. St.: *Am. J. Physiol.* **147**:115, 1946.

Glycine, however, was effective. This is apparently in contrast to the observation of Fishman and Artom,³ who did not observe a beneficial influence of the glycine added to the synthetic diet. *dl*-Serine, however, in a similar manner will damage the kidney only if given by stomach tube or intraperitoneally but not if added to the diet.¹⁷ In addition, the amount of glycine injected was considerably larger.

Of other substances tested, *dl*-alanine, *dl*-threonine and glycollic, butyric and pyruvic acids, likewise were effective in preventing the *dl*-serine-produced renal injury. Pyruvic acid suppressed the renal necrosis induced by *dl*-serine, although it caused a moderate degree of hydropic degeneration in segments of the convoluted tubules located in the outer portions of the renal cortex. *l* (+) Histidine monohydrochloride and lactic acid afforded appreciable, while *l* (+) arginine monohydrochloride and *dl*-valine afforded moderate, protection. Glucose, sodium acetate and sodium chloride were without influence.

The number of substances tested had to be limited, of necessity. However, it is likely that other compounds can be found capable of influencing the renal injury induced by *dl*-serine.

Artom and Fishman postulated that the injurious action of *dl*-serine might be due to a mass action of its unmetabolized molecule or to the accumulation of products of its metabolism.¹⁷ They later found the unnatural *d*-isomer to be injurious, while *l*-serine was not toxic.¹⁸

In view of the accumulation of bisulfite-binding substances in the urine of animals poisoned with *dl*-serine and the evidence brought forward that serine might be converted into pyruvic acid,¹⁹ Fishman and Artom²⁰ considered the accumulation of pyruvic acid in the kidney as a possible cause of the renal injury. However, as previously stated, pyruvic acid has considerable protective action and, although producing some hydropic changes, it does not lead to renal necrosis.

As mentioned before, cystine can be formed from serine in the body of the rat.²¹ Cystine was found to exert a nephrotoxic action if given intravenously to dogs in large amounts.²² It has an aggravating influence on the renal damage caused by choline deficiency.⁶ Neither in rats fed the normal diet nor in those fed the synthetic diet, however, did

17. Artom, C., and Fishman, W. H.: Proc. Soc. Exper. Biol. & Med. **57**:239, 1944.

18. Artom, C.; Fishman, W. H., and Morehead, R. P.: Proc. Soc. Exper. Biol. & Med. **60**:284, 1945.

19. Bernheim, F.; Bernheim, M. D. C., and Gillespie, A. G.: J. Biol. Chem. **114**:657, 1936. Chargaff, E., and Sprinson, D. B.: *ibid.* **151**:273, 1943. Binkley, F.: *ibid.* **150**:261, 1943.

20. Fishman, W. H., and Artom, C.: Proc. Soc. Exper. Biol. & Med. **60**:288, 1945.

21. Stetten.^{4a} Binkley and du Vigneaud.^{4b} Du Vigneaud and others.^{4c}

22. Newburgh, L. H., and Marsh, P. L.: Arch. Int. Med. **36**:682, 1925.

large sublethal amounts of cystine or cysteine produce significant lesions of the kidneys.

A considerable fraction of serine is transformed into glycine in the rat and the guinea pig.²³ Glycine itself is completely innocuous and exerts strong protective action. It is of particular interest that only the naturally occurring *l*-serine is converted into glycine.²³ Similarly only the *l*-isomer can be converted into cystine by liver tissue.^{4b} In view of these facts the first hypothesis of Fishman and Artom appears more likely, namely, that the damaging action of *dl*-serine is due to the presence of the unmetabolized *d*-isomer.

At normal plasma concentrations the reabsorption of amino acids from the glomerular filtrate is practically complete, and only small amounts are excreted in the urine. Elevation of the plasma concentration above the normal by the administration of amino acids leads to an increase in the rate of tubular reabsorption. If the process of reabsorption fails to keep pace with the increased rate of amino acid filtration by the glomeruli, increased amounts are excreted in the urine.²⁴ Pitts^{24b} showed that creatine is reabsorbed by a mechanism similar to that which is active in the absorption of various amino acids. Competition between creatine and amino nitrogen for a common link in the reabsorptive chain leads to the reduction in the amount of creatine absorbed at elevated plasma amino acid levels. Glycine, *dl*-alanine and *l* (+) glutamic acid suppress the reabsorption of creatine. In contrast, *l* (+) arginine does not influence the tubular reabsorption of creatine. On the other hand, glycine was found to depress the reabsorption of *l* (+) arginine.

In the light of these findings it seems possible that the protective action of the various amino acids and other compounds against the nephrotoxic action of *dl*-serine is due to the competitive action of these substances on the reabsorption of the injurious *d*-isomer. Since the damage caused by *dl*-serine occurs only in the terminal segments of the proximal convoluted tubules,¹ it can be assumed that reabsorption of this amino acid occurs predominantly at this location. In contrast, glucose is reabsorbed in the first portions of the proximal convoluted tubules.²⁵

23. Shemin, D.: J. Biol. Chem. **162**:297, 1946.

24. (a) Doty, J. R.: Proc. Soc. Exper. Biol. & Med. **46**:129, 1941. (b) Pitts, R. F.: Am. J. Physiol. **140**:156, 1943; **140**:535, 1944. (c) Goettsch, E.; Lyttle, J. D.; Grim, W. D., and Dunbar, P.; *ibid.* **140**:688, 1944. (d) Eaton, A. G.; Ferguson, E. P., and Byer, F. T.: *ibid.* **145**:491, 1946. (e) Beyer, K. H.; Wright, L. D.; Russo, H. T.; Skeggs, H. R., and Patch, E. A.: *ibid.* **146**:330, 1946. (f) Silber, R. H.; Seeler, A. O., and Howe, E. E.: J. Biol. Chem. **164**:639, 1946.

25. Walker, A. M., and Oliver, J.: Am. J. Physiol. **134**:562, 1941.

SUMMARY

The severe necrotizing nephrosis induced by *dl*-serine can be favorably influenced by several amino acids and related compounds. *dl*-Methionine and glutathione exert a considerable protective influence. This is probably not due to SH groups, since cysteine and thioglycollic acid have only little and 2, 3-dithiopropanol (BAL) has no beneficial effect. *dl*-Alpha-alanine, glycine, *dl*-threonine and glycollic, butyric and pyruvic acids have a considerable protective influence. *l* (+) Histidine monohydrochloride and lactic acid afford appreciable, *l* (+) arginine monohydrochloride and *dl*-valine moderate, protection. Some influence was seen from *l* (+) glutamic acid, while glucose, sodium acetate and sodium chloride were without effect. Neither cysteine nor pyruvic acid nor glycine, substances which can be formed from serine in the body of the rat, cause necrotizing nephrosis.

It is assumed that the beneficial effect of the various amino acids and other substances against the nephrotoxic action of *dl*-serine is due to their competitive suppression of tubular reabsorption of the injurious *d*-isomer.

Case Reports

FIBROMYOSIS UTERI: ENDOMETRIAL TYPE

J. H. HILL, M.D., KANSAS CITY, MO.

A UTERINE growth which resembles adenomyoma but which does not contain glands occurs with considerable rarity. For that reason the study of another case is justified. Goodall¹ called this type of growth "stromatous endometriosis," but the name is somewhat of a misnomer because no glands are seen in this condition. It would seem that the term "fibromyosis uteri, endometrial type" more accurately describes the condition. Frank² suggested the term "fibromyosis" for a similar condition, but this term does not sufficiently indicate the endometrial character of the connective tissue growth.

The first case was described as one of adenomyosis with stroma but no glands by Casler³ in 1920. Between 1937 and 1941 Goodall brought this condition into prominence by his reports of 8 cases. His monograph must be consulted for all his opinions regarding endometriosis. In 1942 Robertson and associates⁴ studied 6 cases of what they described as "benign stromal endometriosis" and a seventh case of growth clearly cancerous in character. They expressed the belief that adenomyosis and stromal endometriosis are variants of the same process but that the lesions of stromal endometriosis, unlike those of adenomyosis, may continue to grow after the cessation of ovarian function. Three cases were reported by Miller and Tennant⁵ in 1944. In 1945 de Carle⁶ reported 3 cases. Quite recently Henderson⁷ has presented a clinical and pathologic study of 7 cases; to this condition he has given the name "endolymphatic stromal myosis." I have personal knowledge of 2 unreported cases, and in the literature are references to 3 other unreported cases. This condition may be somewhat more common than the literature would indicate.

REPORT OF A CASE

E. E. W., a 46 year old white woman, was admitted to the service of Dr. John Ogilvie at the Trinity Lutheran Hospital on May 1, 1946, complaining of *profuse menstrual bleeding* and a lump in the lower part of the abdomen. The

From the departments of pathology of the University of Kansas School of Medicine and the Trinity Lutheran Hospital.

1. Goodall, J. B.: Tr. Am. A. Obst. **50**:192, 1938; J. Michigan M. Soc. **39**:480, 1940; J. Obst. & Gynaec. Brit. Emp. **47**:13, 1940; A Study of Endometriosis, Endosalpingiosis, Endocervicosis and Peritoneo-Ovarian Sclerosis, ed. 2, Philadelphia, J. B. Lippincott Company, 1944.

2. Frank, R. T.: Am. J. Cancer **16**:1326, 1932.

3. Casler, D. B.: Surg., Gynec. & Obst. **31**:150, 1920.

4. Robertson, T. D.; Hunter, W. C.; Larson, C. P., and Snyder, G. A. C.: Am. J. Clin. Path. **12**:1, 1942.

5. Miller, J. R., and Tennant, R.: Am. J. Obst. & Gynec. **47**:784, 1944.

6. deCarle, D. W.: West. J. Surg. **53**:48, 1945.

7. Henderson, D. N.: Am. J. Obst. & Gynec. **52**:1000, 1946.

menorrhagia had begun seven months before admission and had become progressively more severe. One month before admission she suffered abdominal pain described by her as like "a green apple bellyache." At that time she noticed a lump in the lower part of the abdomen. She has 3 children aged 23, 21 and 18 years. Significant physical findings consisted of a firm, movable, nontender mass "about the size of a grapefruit," the upper surface of which could be palpated half way between the symphysis pubis and the umbilicus. Laboratory examination revealed moderate secondary anemia. The clinical diagnosis was uterine myoma. A supracervical amputation of the uterus was performed, with convalescence uneventful. When seen eight months after operation the patient was well except for hot flushes and nervousness requiring estrogenic therapy.

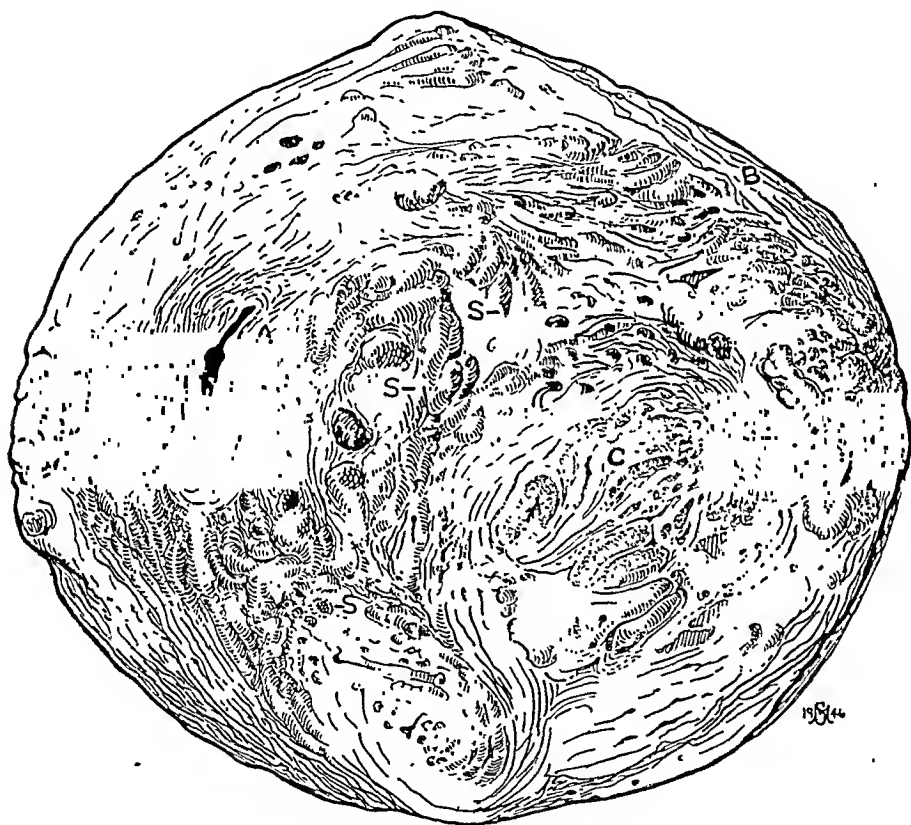


Fig. 1.—Drawing of a horizontal cross section of the uterus illustrating the assymetric enlargement of the uterus caused by intramural masses of stromal cells. *A* indicates the uterine cavity lined by unchanged mucosa; *B*, the narrow rim of compressed myometrium; *C*, an irregular focus of necrosis; *S*, stromal cell masses. $\times 0.92$.

The specimen consisted of a spherical uterine tumor mass approximately 12 cm. in diameter. Gross section revealed the uterine cavity to be small and deflected far to one side by a tumor-like growth of one wall. This enlargement consisted of interlacing muscle bundles enclosing islands of pale, soft cellular tissue. In the fixed material (fig. 1) the muscle bundles contracted and the rubbery cellular islands bulged above the cut surface. Toward the center of the thickened wall was an irregularly shaped, reddish brown mottled area of softening approximately 3 cm. in diameter. The endometrium averaged 4 mm. in

thickness and had a smooth surface. Attached to the uterine fundus was a pedunculated subserous fibromyoma 4 by 3 by 3 cm. in size. The uterine tubes were congested. The ovaries were not enlarged; both were firm and contained numerous scars, and one bore a mature corpus luteum.

Microscopic sections from the thickened uterine wall showed broad masses of cells infiltrating between the bundles of smooth muscle (fig. 2 *A*); in places the cell masses grew into and along dilated lymphatic channels (fig. 2 *C* and *D*). The cells tended to be uniform in size and shape with oval, moderately vesicular nuclei that occasionally showed mitotic figures; the cytoplasm was so scanty as

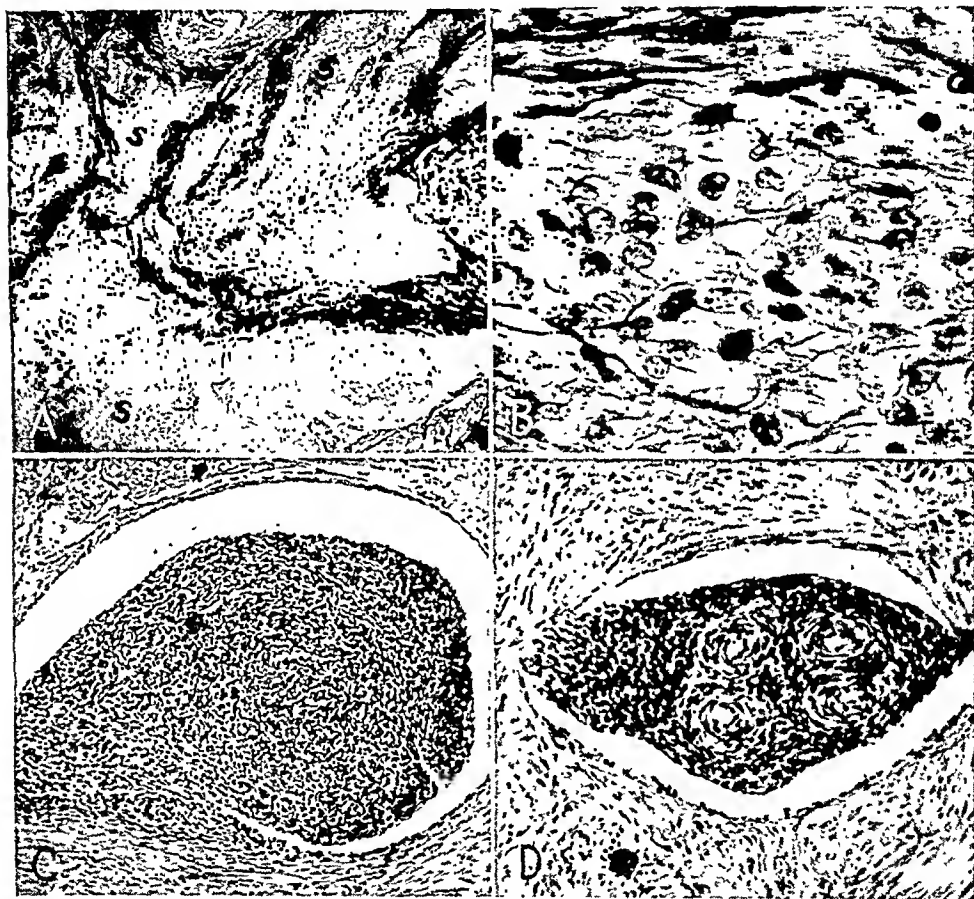


Fig. 2.—*A*, low power photomicrograph of a section of thickened uterine wall, illustrating the diffuse infiltrative type of stromal cell growth. Note muscle bundles (*m*) surrounded by stromal cell masses (*s*). Aniline blue-azocarmine; $\times 65$.

B, photomicrograph showing diffuse network of reticulum fibers more or less intimately related to each stromal cell. Note the endothelial tube (*a*) and the uniformity of the nuclei of the stromal cells. Fine short strands of collagen can be demonstrated in the field with aniline blue and light green. Wilder's reticulum stain. $\times 591$.

C, photomicrograph showing a mass of stromal cells in a dilated lymph vessel. Note that the mass is broadly attached to the vessel wall and that the intrinsic vascular tubes are apparently connected with the lumen of the lymph vessel. Hematoxylin-eosin; $\times 85$.

D, photomicrograph showing four arteriole-like vessels contained in an endolymphatic mass of stromal cells. The surrounding myometrium is hyperplastic. Hematoxylin-eosin; $\times 110.5$.

to be almost invisible: Reticulum stains revealed abundant reticulum formation with delicate argyrophilic fibrils intimately related to and surrounding each cell (fig. 2 *B*). Sections stained by the Masson technic revealed occasional inconspicuous collagen fibrils chiefly about the intrinsic vessels of the tumor. Elastic fibers were not demonstrated. The cells making up the invading masses were apparently derived from the endometrial stroma.

The vascular component of the cell masses was of considerable interest. Some vessels were obviously myometrial arteries that had been surrounded by the growing cells. Present throughout the specimen were small arteriole-like blood vessels having a concentrically arranged sheath of stromal cells. Reticulum stains showed a perivascular condensation of argyrophilic fibrils, some of which also stained for collagen; smooth muscle cells could not be demonstrated. These arteriole-like vessels sometimes occurred in small groups accompanying the growing cell masses into and along dilated lymph vessels (fig. 2 *C* and *D*); such vessels were formed by the stromal cells themselves. Other irregular, slightly dilated vascular spaces lined by flattened endothelium were present; those containing erythrocytes were considered to be venules, while the empty ones were lymph vessels.

Sections of the softened areas revealed foci of necrosis. Close to the foci of necrosis the stromal cells showed swelling of the cytoplasm and nuclear change, while other areas showed much interstitial edema and separation of the cells, resulting in a myxoma-like appearance. In some places the stromal cells were indistinct except for those around blood vessels, producing a perithelioma-like appearance.

Sections from the relatively intact, though thickened, uterine wall showed early secretory phase endometrium, while the underlying myometrium showed mild hyperplasia but no evidence of stromal cell growth. Section of one cornu showed slight adenomyosis of the usual type. Section of the broad ligaments revealed no evidence that tumor cells were extending beyond the limits of the uterus.

Some portions of the neoplastic tissue of the uterus showed rather thick bundles of moderately enlarged smooth muscle cells; the microscopic appearance together with the gross anatomy indicated myometrial hyperplasia.

The pathologic diagnosis was: fibromyosis uteri; endometrial type; focal cornual adenomyosis; subserous fibromyoma.

COMMENT

After studying this case and those previously reported, I do not believe that these cases should be called instances of endometriosis, because the stromal cell growth does not contain glands and in some cases at least the growth persists and causes death after ovarian function has been abolished. I have employed Frank's term "fibromyosis" to describe the growth observed in the present case, adding the phase "endometrial type" to indicate the specific endometrial stroma type of fibrogenic growth which characterizes this condition. While the stromal cells are actively reticulogenic and form only collagen about the intrinsic blood vessels, I think the "fibro-" is justified in view of the intimate connection known to exist between the argyrophilic reticulum and collagen; furthermore, McDonald and associates⁸ concluded that endometrial stroma sarcoma was to be viewed as a variety of fibrosarcoma

8. McDonald, J. R.; Broders, A. C., and Counseller, V. S.: Surg., Gynec. & Obst. **70**:223, 1940.

since the growth sometimes forms collagen. The "myosis" is necessary to indicate the associated hyperplasia of smooth muscle which accompanies the stromal cell masses. Since the stromal cell of the endometrium is unique, I feel that the qualifying phrase "endometrial type" is required to indicate the specific cell type comprising the fibrogenic tissue.

As used by Goodall the term "endometriosis" implies a growth that may be composed of glands and stroma or of stromal cells alone. Ordinarily the term "endometriosis" implies the presence of both glands and stroma, their proportions varying within considerable limits; in the absence of either stroma or glands it is not usually believed that endometriosis is present. Furthermore, there is insufficient evidence to overthrow the view that the epithelium plays the dominant and formative role while the stroma plays a subordinate role. Novak⁹ stated that the endometrial stroma was to be looked on as a satellite tissue appearing as the natural accompaniment of celomic epithelium which has reached a certain degree of differentiation. The differentiation existing between the stroma and the epithelium of endometrium, according to Gilbert,¹⁰ is of a different order from that between the stroma and the epithelium of other organs; this together with the fact that they are both sensitive to endocrine secretions accounts, in his opinion, for some of the peculiarities seen in endometrial stroma growths. Proof that the normal form and function of the epithelium require contact or working together with the endometrial stroma has been offered by de Josselin de Jong.¹¹ He showed that when the glandular epithelium came in contact with fibrous connective tissue the epithelial cells became flattened so as to resemble endothelium. A study of decidua shows that the epithelium may become so flattened as to cause difficulty in distinguishing the glands from lymph channels or from venules.¹² These two observations indicate that the kind of supporting stroma may radically affect the appearance of the glandular epithelium, causing it to resemble endothelium. With this in mind I examined the sections of the present specimen in an effort to determine whether or not some of the endothelial-lined spaces could represent altered endometrial glands and thus allow the growth to be classified as a sort of atypical endometriosis. In this I was unsuccessful; that is, transition between glandular epithelium and endothelium could not be made out.

I believe these stromal cell growths arise from mesenchymal cell rests laid down during the development of the müllerian duct structures. Evidence that patches of mesenchymal cells do occur in various locations in the female pelvis and that they are sensitive to endocrine stimulation is afforded by a study of ectopic decidua islands.¹³ I believe that these rests may occur in the myometrium and that an endocrine stimulus accounts for hyperplasia in the direction of the endometrial stromal cell and for the myometrial hyperplasia. The resultant

9. Novak, E.: *Am. J. Obst. & Gynec.* **12**:484, 1926.

10. Gilbert, J. B.: *Clin. Proc.* **2**:214, 1943.

11. de Josselin de Jong, R.: *Schweiz. med. Wchnschr.* **65**:197, 1935.

12. Novak, E.: *Gynecological and Obstetrical Pathology*, Philadelphia, W. B. Saunders Company, 1940, p. 123.

13. Novak,¹² p. 124.

growth is to be looked on as a hamartoma possessing a limited capacity for growth; it is probable that the hyperplasia may sometimes assume autonomous growth resulting in what amounts to a type of low grade fibrosarcoma.

In diagnosis the growth must be differentiated from endometrial sarcoma, leiomyosarcoma and anaplastic carcinoma of the endometrium. It may be excluded on the finding of polymorphism, numerous mitotic figures and destructive invasion of neighboring tissues.

The 3 cases reported by Frank under the title "‘Fibromyosis’: An Unclassified Plexiform Endolymphatic Proliferation of the Uterus" differ from the present case in that the tumor cells were largely confined within lymph channels and extended beyond the limits of the uterus. However, I think that both processes are basically similar. Those patients in whom all the growth is contained within the uterus are probably cured by operation, while those showing extension into the parametrium will probably suffer recurrence. It must be emphasized that the long latent period reported in some of the cases showing recurrence requires that ten to twenty year follow-up studies be made before statements of cures may be regarded as conclusive.

SUMMARY

A case of fibromyosis, endometrial type, is reported. It is believed that classifying growths of this type as variants of endometriosis is unwarranted. The term "fibromyosis," suggested by Frank, is more desirable when coupled with the qualifying phrase "endometrial type."

Obituaries

OSCAR T. SCHULTZ, M.D.

1877-1947

Oscar T. Schultz lived, until his college years, in the small farming town of Mount Vernon, Ind. He was the first of four children born to Dr. O. T. Schultz and Louise Pfeffer Schultz and was early introduced to the atmosphere of a country general practice. Dr. Schultz Sr. was from a long line of doctors extending over several generations and thus it was natural for his son to take up the study of medicine. The precollege school education of Dr. Schultz Jr. was supplemented by a considerable degree of discipline and training supervised by his father, who taught him Greek and encouraged the use of German, which was spoken in that family and by O. T. from early childhood, and which later proved to be a great advantage. Dr. Schultz Sr. recognized in his son certain retiring and shy qualities and encouraged him to pursue the nonclinical studies in medicine rather than to engage in practice. Dr. Schultz Jr. was a "Hoosier" until he went to the Johns Hopkins University to study medicine, for he acquired his A. B. degree at Indiana University in 1897. Two years elapsed before he entered Johns Hopkins, in 1899—two years in which he served as a sergeant in the Spanish-American War.

After graduating from Johns Hopkins in 1903, he spent a few months at Columbia University and at the Brooklyn Institute. However, his career was firmly established in Cleveland, where he successively served as resident pathologist in the Charity Hospital and demonstrator in pathology and protozoology at Western Reserve University, then as instructor and lecturer, and finally as associate professor in pathology from 1910 to 1913 at the same university. Meanwhile he had married Irene Throop, with whom he had grown up in Mount Vernon, and with his wife had three children: Dr. Katherine, Louise and Oscar Jr. In 1913 he became professor of pathology and bacteriology at the University of Nebraska Medical School and remained there until 1916. In that year he became director of the Nelson Morris Institute of Michael Reese Hospital, Chicago, remaining in that capacity until 1929. This period was interrupted by a two year service in the Army as captain in the medical corps, a considerable portion of which time was spent in Europe. In 1928 he suffered a severe illness, which resulted in a serious and permanent reduction of vision. Nevertheless in 1929 he undertook the directorship of the laboratories at the St. Francis

Hospital in Evanston and was there in that capacity when his final illness began in November 1943. He experienced at that time a cerebral



OSCAR T. SCHULTZ, M.D.

1877-1947

vascular accident which resulted in hemiplegia and total disability lasting until his end in March 1947.

O. T. was a member or fellow of many medical societies—the American Association for the Advancement of Science, the American Association of Pathologists and Bacteriologists, the Society for Experimental Biology and Medicine, the American Medical Association, the Institute of Medicine of Chicago and the Chicago Pathological Society, which he served as president in 1922. During the ten years prior to his illness he became keenly interested in medicolegal matters and was an active member of the Chicago Medico-Legal Society and the Chicago Academy of Criminology. Among his other activities, he was editor of the *Cleveland Medical Journal* from 1910 to 1913 and was visiting pathologist to Columbia Hospital in Milwaukee from 1930 to 1932. He became a member of the editorial board of the *ARCHIVES OF PATHOLOGY* in 1927 and remained on the board until 1944.

Dr. Schultz's contributions to the literature may be divided into three groups. During his years at Cleveland he contributed papers on syphilis, on topics in the fields of dermatopathology and protozoology and, with Professor Howard, wrote a monograph on tumor cells which was published under a grant from the Rockefeller Institute as one of the series of monographs in medical research. While at Michael Reese Hospital he contributed chiefly to Abt's system of pediatrics—articles on pathologic involvements of the lung and of the kidney and in particular a long section on tumors of infancy and childhood. His third period was from 1929 to 1940, in which he contributed a comprehensive review and critical analysis of the coroner system in the United States, the material concerning which was published in monograph form by the National Research Council, in addition to several shorter papers on the same subject and concerned with limited phases published in different medical journals.

Essentially O. T. was not a research investigator, but those of us who worked with him and knew him well appreciated his great capacity as a pathologist and his rather remarkable memory of medical literature. He was a teacher not for audiences but rather for intimate students and co-workers and, by example, taught the virtues and practices of intellectual honesty.

A modest man tending to the shy and retiring manner, he was not one to air his burdens and problems, but always appeared to be urbane and at ease. To those few who were intimate with him he was a warm and sympathetic friend. I regarded him as a highly civilized person, entirely free of pettiness and prejudice. He was one of that all too rare kind not affected by current popular hysterias, not given to hasty, impulsive expressions. He was good company; over a lunch table or in intimate evening gatherings he contributed to lusty discussion in many fields. He had a genuine fondness for music and a lively

appreciation for "good living." O. T. served his profession with great devotion and conscientiousness. He was a source of advice and assistance, given without stint and in full measure of his capacities. All who worked with him or under his direction will remember him with affection—for his kindness, courtesy and respect for human dignity. Surgeons who witnessed his great skill with tissues will remember him for his remarkable accuracy of diagnosis, maintained in spite of handicaps of vision and health.

NORBERT ENZER, M.D.

Notes and News

Awards.—Selman A. Waksman has received the 1947 Passano Foundation Award of \$5,000 for his research in the field of antibiotics culminating with his discovery of streptomycin. The Passano Foundation, established in 1943 by the Williams & Wilkins Company, medical publishers, Baltimore, provides the award to encourage medical research, especially that which has clinical application.

Carl F. Cori, Washington University School of Medicine, has been awarded \$5,000 by the National Science Fund of the National Academy of Sciences for his contributions to knowledge of sugar metabolism.

Deaths.—Sir Almroth Wright, 85, fellow of the Royal Society and former director of the Institute of Pathology, St. Mary's Hospital, London, died on April 30. He originated systematic inoculation to prevent typhoid fever, was a pioneer in therapeutic inoculation and developed methods of measuring the effects of bacterial vaccines.

C. A. Hospers, pathologist to Holy Cross and South Chicago Community hospitals, died of coronary thrombosis, February 20, aged 42. He served in the war as lieutenant colonel in the Medical Corps, the Army of the United States.

Postgraduate Courses in Pathology.—The medical extension at the University of California Medical Center announces the following courses:

1. Gynecologic and obstetric pathology, and the smear technic as it pertains to cancer of the uterus, the stomach, the urinary tract and the lungs, Aug. 4 to 15, inclusive, 1947, daily, 9 a. m. to 4:30 p. m. This course is limited to 75. Each registrant is to furnish his own microscope, of good quality. Either the morning or the afternoon session may be taken alone, or the two may be taken together. It is suggested that experience in the use of the microscope and in the diagnosis of cancer in general will be an essential prerequisite if one is to obtain proficiency and confidence in the judgment of the smear preparations. This course is designed to fulfil two needs: (a) requests from physicians who wish to prepare for the American Board Examinations and (b) requests from pathologists, cytologists, researchers and all others who are interested in learning to use the smear technic for the diagnosis of cancer occurring in various parts of the body.

2. Obstetric and gynecologic pathology, Sept. 1 to 5, 1947, 9 a. m. to 1 p. m. and 2 to 5 p. m.

For information write to Dr. Stacy Mettier, University of California Medical Center, San Francisco 22.

Books Received

RECENT ADVANCES IN CLINICAL PATHOLOGY. By Various Authors. Produced under the Auspices of the European Association of Clinical Pathologists. General Editor, S. C. Dyke, D.M., F.R.C.P. Section Editors: R. Cruickshank, M.D., F.R.C.P., bacteriology; E. N. Allott, B.Ch., F.R.C.P., biochemistry; B. L. Della Vida, M.D. (Rome), hematology and cytology; A. H. T. Robb-Smith, M.D., histology. Pp. 468, with 34 plates and 19 text figures. Price \$5.50. Philadelphia and Toronto: The Blakiston Company, 1947.

During the war, medical refugees in Great Britain took an active part in building up a strong service in clinical pathology. The European Association of Clinical Pathologists was organized. At the meeting of this association in Oxford in July 1944 it was decided to place on record recent additions to the practice of clinical pathology in Great Britain. The result is the book now being reviewed. Forty laboratory workers have written about that many short articles on topics of practical interest fairly evenly distributed in sections on bacteriology, biochemistry, hematology and cytology and histology. The section on histology is devoted mainly to microscopic diagnosis of tumors. It is illustrated by some 30 plates. Aspiration biopsy is described in detail. The book will be of interest to clinical pathologists in general and should be available in every clinical laboratory.

ATLAS OF HISTOPATHOLOGY OF THE SKIN. G. H. Percival, M.D., Ph.D., F.R.C.P.E., D.P.H., Grant professor of dermatology, University of Edinburgh; A. Murray Drennan, M.D., F.R.C.P.E., F.R.S.E., professor of pathology, University of Edinburgh, and T. C. Dodds, F.I.M.L.T., F.I.B.P., F.R.P.S., laboratory supervisor, department of pathology, University of Edinburgh. Pp. 494, with 376 photomicrographs in color. Price \$16. Baltimore: Williams & Wilkins Company, 1947.

The microscopic changes of the more common diseases of the skin are illustrated by photomicrographs in color made by the Finlay process. The text consists of some 40 pages of concise explanations, and there are no references to the literature. The classification is on a clinical basis. Nevus is given a wider scope than usual: "a naevus is a circumscribed hyperplasia or tumour formation which has its origin in aberrant embryological structures or 'rests.' Naevi may be congenital or may appear at any age and are of almost universal occurrence." They are grouped in the atlas as derived from the epidermis, epidermal appendages, collagen and vasoformative cells. The illustrations are well made and clearly described. Most of the sections were stained with hemalum and eosin. Space and paper could have been saved by using both pages of the leaves. The atlas is intended mainly for advanced students of dermatology, but it will be useful to any one interested in cutaneous histopathology.

PATHOLOGY IN SURGERY. By N. Chandler Foot, M.D., professor of surgical pathology, Cornell University Medical College, and surgical pathologist, New York Hospital. Pp. 512, with 368 illustrations in black and white and 20 subjects in full color on 10 plates. Price \$10. Philadelphia: J. B. Lippincott Company, 1945.

THE ROCKEFELLER FOUNDATION. A REVIEW FOR 1946. By Raymond B. Fosdick, president of the Foundation. Pp. 64, illustrated. New York: The Rockefeller Foundation.

PHYSICIAN'S HANDBOOK. John Warkentin, Ph.D., M.D., and Jack D. Lange, M.S., M.D. Fourth edition. Pp. 282, illustrated. Price \$1.50. Chicago: University Medical Publishers (P. O. Box 5067), 1946.

THE MURINE TYPE OF TUBERCLE BACILLUS (THE VOLE ACID-FAST BACILLUS). By A. Q. Wells. With Notes on the Morphology of Infection by the Vole Acid-Fast Bacillus by A. H. T. Robb-Smith. Medical Research Council, Special Report Series no. 259. Pp. 48, with 13 plates. Price, 2 shillings. London: His Majesty's Stationery Office (New York: British Library of Information), 1946.

THE RELATION OF DISEASES IN THE LOWER ANIMALS TO HUMAN WELFARE. By William A. Hagan, Herald R. Cox, William H. Feldman, I. Forest Huddleson, Harald N. Johnson, Raymond A. Kelser, Joseph V. Klauder, Karl F. Meyer, C. D. Stein and Willard H. Wright. *Annals of the New York Academy of Sciences*, Volume XLVIII, art. 6, pages 351-576. New York: New York Academy of Sciences, 1947.

PRACTICAL PHYSIOLOGICAL CHEMISTRY. By Philip B. Hawk, Ph.D., president, Food Research Laboratories, Inc., Long Island City, N. Y.; Bernard L. Oser, Ph.D., director, Food Research Laboratories, Inc., Long Island City, N. Y., and William H. Summerson, Ph.D., associate professor of biochemistry, Cornell University Medical College, New York. Twelfth edition. Pp. 1323, with 329 illustrations. Price \$10. Philadelphia: Blakiston Company, 1947.

The new twelfth edition of "Practical Physiological Chemistry" has continued its policy of previous editions of expanding the clinical and theoretic phases of the book without detracting from its practical features as a laboratory guide to physiologic and nutritional chemistry. Whether by doing so Dr. Hawk and his new associates, Drs. Oser and Summerson, have finally succeeded in making the book a satisfactory textbook for medical students cannot easily be determined. At any rate, this edition is undoubtedly more acceptable in this regard than any previous one.

Though the book retains the format of its predecessors—as symbolized by the reinsertion of the unrealistic color plates of absorption spectrums in the frontispiece—so many new discussions have been added and so many up-to-date references inserted as to make it refreshingly new. Such items as the chemical formula of penicillin, microbiologic assays of antibiotics and amino acids, steroid hormones, isotopes, photometric and flame photometers and many others described only yesterday offer a delightful surprise. Similarly, the discussion of the intermediate metabolism of protein, carbohydrate and lipids is up-to-date. The objective chemical discoveries of the last few years are stressed at the expense of the more controversial physiologic theories. The explanations are lucid and well interspersed with formulas and diagrams.

The chapters on blood and urine analysis are strengthened by an excellent discussion of photoelectric photometry and of the available instruments. But the choice of analytic methods and their description frequently show the bias of tradition and a denial of the modern universality of photoelectric technics. In these chapters there seems to be still room for the criticism of early editions, that the laboratory procedures are at times offered without the benefit of the personal experience of the authors with their limitations.

As a reference book of modern practical physiologic chemistry, this book has a place in the library of students, clinicians or experimental workers who have any contact with the laboratory phases of biochemistry.

MEDICINE IN THE CHANGING ORDER. Report of the New York Academy of Medicine on Medicine and the Changing Order. Volume 8. Pp. 240. New York: The Commonwealth Fund, 1947.

The Committee of the Academy was appointed in December 1942 "with unlimited latitude to study present trends in medicine." This committee was "composed of 33 physicians and 17 representatives of allied professions and lay persons. The physicians were chosen because of their interest in medical education and public health. There was strong representation in the field of nursing and dentistry. The lay members were selected from the fields of the ministry,

the law, social welfare, the hospital, insurance, labor and industry." In this study "it was agreed that we should solicit information and opinion from a wide variety of authorities. Those consulted represented every shade of economic, social and political conviction that might have a bearing on medical care."

The growth of medicine in America is traced from colonial to modern times, in parallel with coincident economic changes, the increase in population and its shifts from rural to urban areas, and the changes in trends of social thinking. Medical care in urban and rural areas, the quality, cost and distribution of medical care, and methods for its improvement are discussed.

With respect to medical insurance, whether voluntary or compulsory, the committee concludes that while voluntary insurance will of necessity grow more slowly, it can be adapted in its several forms more readily to the varying problems of different communities and areas. The committee concludes that national compulsory medical insurance "would not and could not realize the promises made for it and would inevitably create new and formidable evils of its own." The unfavorable experience with compulsory medical insurance in England with its mounting costs and poor quality of service are described.

The historical approach is utilized in the report, which is factual, temperate and judicial in its treatment of the extremely complicated problem of how to improve medical care.

INTRANUCLEAR INCLUSION BODIES IN THE KIDNEYS OF WILD RATS

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INTRANUCLEAR inclusion bodies have been noted in the renal cells of man and in those of a wide variety of mammals and birds.¹ Although cellular inclusions are accepted as evidence suggestive of the activity of a virus, efforts to recover a causative agent from inclusion-bearing renal tissues have to the present been unsuccessful. Accordingly, when we found renal tubular cells of wild rats captured in Rochester, N. Y., to contain intranuclear inclusion bodies of Cowdry's² type B, experiments were designed with a view to learning (a) the incidence of the occurrence of the intranuclear inclusion bodies in wild rats and the intensity of involvement of the renal tubular cells, (b) whether the causal agent of the intranuclear inclusion bodies is of an infectious nature and (c) whether similar intranuclear changes could be produced by employing a foreign substance such as was used by Olitsky and Harford³ to induce the formation of inclusion bodies. This paper reports the results of our studies.

Intranuclear inclusion bodies were first recorded as being present in the renal cells of rats by Hindle and Stevenson.⁴ Hindle⁵ in further studies observed intranuclear inclusion bodies to be present almost invariably in the kidneys of London sewer rats, whereas they were absent from

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A preliminary report was published in the Proceedings of the Society of American Bacteriologists (J. Bact. **45**:80, 1943).

1. (a) For related bibliography, see Findlay, G. M., in Doerr, R., and Hallauer, C.: *Handbuch der Virusforschung*, Berlin, A. Hirschwald, 1938, pp. 337-339. (b) Kinney, T. D.: *Am. J. Path.* **18**:799, 1942.

2. Cowdry, E. V.: *Arch. Path.* **18**:527, 1934.

3. Olitsky, P. K., and Harford, C. G.: *Am. J. Path.* **13**:729, 1937.

4. Hindle, E., and Stevenson, A. C.: *Tr. Roy. Soc. Trop. Med. & Hyg.* **23**:327, 1929-1930.

5. Hindle, E.: *Nature*, London **129**:796, 1932.

country rats. Hindle and Coutelen,⁶ in Paris, and Rector,⁷ in St. Louis, found similar inclusions in the kidneys of wild rats. Cowdry² and Cowdry, Lucas and Fox⁸ concluded from morphologic studies of sections provided by Hindle⁹ that these inclusions belong to type B. They stated further concerning these bodies, "we entertain the possibility of virus action and await experimental proof." Rector⁷ attempted experimentally to produce similar inclusion bodies in 8 normal white rats by injecting a Berkefeld-N filtrate prepared from inclusion-containing tissue derived from 2 wild rats. However, when these 8 white rats were killed at intervals up to twenty days after injection and their kidneys examined microscopically, no inclusion bodies were found.

MATERIALS AND METHODS

Rats.—One hundred and thirty-nine wild rats (*Rattus norvegicus*) were utilized to provide kidneys for histologic study and for tissue transfer. The source of these rats was as follows: Rochester provided 117 rats, of which 65 were caught in or near the animal house of the medical school, to which a seasonal immigration from outlying districts occurs, and 52 were captured in parts of the city where they had ready access to sewers and refuse; 5 came from farms in upstate New York; 12 were captured on the water front in San Francisco; 5 were sent from Denver.

Sixty-two normal albino rats (*Rattus norvegicus*) of the Wistar strain were used. Of these rats, 33 were employed as recipients for suspensions of renal tissues known to contain many inclusion bodies, and 29 served as hosts for injections of aluminum hydroxide gel.

Preparation of Tissues for Microscopic Examination.—Immediately after a rat was killed, its kidneys were removed under aseptic precautions and hemisectioned longitudinally. One half of each kidney was prepared for microscopic study either by fixation in Zenker's (5 per cent acetic acid) fluid for subsequent staining with hematoxylin and eosin and by Giemsa's method or by employing the frozen section technic and staining the sections with hematoxylin and eosin. The other half of each kidney was used for transmission to animals and for bacteriologic studies.

Preparation of Suspensions for Infection.—Suspensions of the renal tissues of wild rats were prepared for injection into albino rats according to the following technic. Kidneys immediately after their removal from each wild rat were made ready for microscopic examination by employing the frozen section technic and staining the sections with hematoxylin and eosin. Only tissues found on microscopic examination to contain numerous inclusion bodies (4 plus)¹⁰ were accepted

6. Hindle, E., and Coutelen, F.: *Compt. rend. Soc. de biol.* **110**:870, 1932.

7. Rector, L. E.: *Proc. Soc. Exper. Biol. & Med.* **34**:700, 1936.

8. Cowdry, E. V.; Lucas, A. M., and Fox, H.: *Am. J. Path.* **11**:237, 1935.

9. These histologic sections were prepared from tissues removed from rats in England and sent by Dr. E. Hindle to Dr. E. V. Cowdry (*Am. J. Path.* **11**:237, 1935).

10. The stained sections were systematically examined, mechanical stage, a 10 × ocular, and 4 mm. and 1.8 mm. objectives being employed. The intensity

as satisfactory for passage. Such tissues were triturated with alundum in Locke's solution to yield a 10 per cent suspension. Meat extract broth was substituted for Locke's solution when filtration experiments were contemplated. These suspensions were centrifuged horizontally at 1,500 revolutions per minute for thirty minutes, and each supernatant fluid directly, or after filtration, was used undiluted in the inoculation of albino rats.

Alumina gel type C was made according to the method of Willstätter and Kraut¹¹ as modified by Sabin.¹²

Preparation of the Albino Rats Used as Recipients for Inclusion-Containing Tissue.—The possibility that naturally acquired inclusion bodies might be present in the renal cells of the white rats employed as recipients for test materials was considered. Accordingly, the left kidney from each of 33 white rats was removed under aseptic precautions¹³ through a curved incision that paralleled the twelfth rib in the left lumbar region. Tissue sections from each kidney were prepared and studied microscopically. Inclusion bodies were not found in a single kidney. Accordingly, when all of these albino rats had made an uneventful recovery from nephrectomy ten to twenty days later, they were inoculated with a suspension of inclusion-containing tissue.

DESCRIPTION OF THE NATURALLY OCCURRING DISEASE

Our description of the naturally occurring disease¹⁴ is necessarily inadequate because it was impossible to keep wild rats under observation for prolonged periods.

Observations on the naturally occurring disease were limited to inspection of a single group of 10 rats during a three week period and of the remaining 129 rats immediately following capture.

The wild rats whose kidneys contained many inclusion bodies at the time of their capture were indistinguishable on gross inspection from rats whose kidneys contained no inclusion bodies. It was noted, however, that inclusions were absent from immature rats that were less than half grown and from rats captured on farms. On the other hand, battle-scarred males, obviously old, had kidneys that contained the greatest number of inclusion bodies. It is noteworthy that most of the old rats also yielded on cultures of tissues *Leptospira icterohaemorrhagiae*¹⁵ and *Salmonella typhimurium*. Only rarely were rats found that were not well nourished and in excellent physical condition. It was impossible in

of involvement as shown by the number of cells containing intranuclear inclusion bodies in each high power field was designated as follows: 0 = no inclusion bodies, 1+ = 0 to 1 inclusion body, 2+ = 2 to 6 inclusion bodies, 3+ = 6 to 10 inclusion bodies, and 4+ = 10 or more inclusion bodies.

11. Willstätter, R., and Kraut, H.: Ber. ü. d. chem. Gesellsch. **56**:149, 1923.

12. Sabin, A. B.: J. Exper. Med. **56**:307, 1932.

13. Ether anesthesia was employed for all operative procedures.

14. In the present paper the term "disease" is used to designate a morbid condition that is manifested by characteristic intracellular alterations in the renal tubular cells.

15. Syverton, J. T.; Stiles, W. W., and Berry, G. P.: J. Bact. **36**:285, 1938.

the latter group to relate their poor physical condition to the bacteriologic and histologic observations.

These observations led us to believe that if the inclusion bodies were associated with an infection, the infection was inapparent. The association in many animals of inclusion disease and either leptospiral infection or salmonellosis, or both, suggested that these two diseases also were present as inapparent infections in hosts that served as carriers.

PATHOLOGIC AND HISTOLOGIC EXAMINATIONS

Since it was apparent early in the present studies that there were no external signs diagnostic of the disease, it was the histopathologic examination of renal tissue that determined when a diagnosis of renal inclusion

TABLE 1.—*Incidence of Renal Inclusion Disease in Two Hundred and One Rats (Rattus norvegicus)*

Type of Rat	Rats Whose Renal Cells Contained Inclusion Bodies		Rats Whose Renal Cells Did Not Contain Inclusion Bodies	
	Number	Per Cent	Number	Per Cent
Wild.....	68	49	71	51
Albino.....	0	0	62	100

disease was made. Accordingly, the kidneys from each of 139 wild rats and 62 stock albino rats of the Wistar strain were examined macroscopically and microscopically for pathologic alterations.

The kidneys from wild rats showed wide variation in their external appearance. Young wild rats and stock albino rats yielded kidneys from which the capsules separated readily, leaving uniformly smooth cortical surfaces. In contrast

TABLE 2.—*Intensity of Involvement in Sixty-Eight Wild Rats Proved to Have Renal Inclusion Disease*

Involvement Graded According to the Approximate Number of Inclusion Bodies per High Power Field	Rats	
	Number	Per Cent
1+ (from none to 1).....	22	32
2+ (from 2 to 6).....	17	25
3+ (from 6 to 10).....	14	20
4+ (10 or more).....	15	22

to these findings, old wild rats almost without exception had kidneys that presented cortical surfaces with extensive focal scarring following removal of the adherent capsules. The scars were few for the most part, irregularly distributed to involve all parts of the cortex, variable in size and pyramidal in shape. On longitudinal hemisection the scars were found to extend into the medullary substance.

One or more sections from each kidney were examined microscopically to determine whether intranuclear inclusion bodies were present and, if so, the relative number, the location and the character of any

attendant lesions. The results of the microscopic examination of renal tissue from each rat are summarized in tables 1 and 2.

It can be seen that intranuclear inclusion bodies were present in renal tissue from 68 (49 per cent) of the 139 wild rats studied. The numbers of inclusion bodies were approximated by utilizing an arbitrary scale ranging from 1 plus to 4 plus.¹⁰ As may be noted in table 2, this rough determination of the intensity of occurrence of inclusion bodies resulted in the finding that there was about the same number of hosts for each grade. The absolute numbers of inclusions ranged from 1 to 75 per high power field.

In a single microscopic field as many as 40 per cent of the epithelial cells lining the convoluted tubules contained the inclusion bodies. On the other hand, the inclusion bodies were not found in the epithelial cells either of the collecting tubules or of the capsules of Bowman. In most instances the inclusion occurred as a single, homogeneous, smooth-appearing, pinkish purple-staining body situated within, and largely replacing, the nucleoplasm (fig. 1). More usually, the basophilic chromatin was concentrated around the inclusion body, and the relatively small basophilic nucleolus had migrated to the nuclear membrane (fig. 2). In some cells, however, an areola surrounded the inclusion body, which exhibited marked acidophilic properties as shown by an intense scarlet-red coloration, and its presence was associated with margination of the basophilic chromatin network and nucleolus (fig. 3). Inclusion-bearing cells frequently were greatly hypertrophied, being several times normal size (figs. 4 to 6). These host cells stood out in contrast to normal cells and to other cells of normal size that contained inclusion bodies.

The concomitant renal lesions were variable. Grossly visible depressions and scars were found to correspond to areas of chronic pyelonephritis as shown by cellular infiltration, varying amounts of fibrosis and damage of glomeruli and tubules. The chronic inflammatory cells were predominantly lymphocytes but included many plasma cells and monocytes. (A representative section is pictured in figure 7.) These findings were without apparent relation to the presence of intranuclear inclusion bodies.

The histologic examination of tissues from 139 wild rats and from 62 laboratory albino rats made it apparent that the only distinctively diagnostic finding in renal inclusion disease was the presence in normal to greatly enlarged renal epithelial cells of a homogeneous, hyalin-like, pinkish purple circular body replacing the nucleoplasm and basophilic chromatin network.

BACTERIOLOGIC STUDIES

Because of the possibility that inclusion bodies might be associated regularly with a cultivable agent, bacteriologic studies were made in an attempt to isolate from renal tissue known to contain inclusion bodies, bacteria, fungi and pleuropneumonia-like organisms.

A triturated suspension of kidney tissue from each of the first 12 rats studied was cultured by inoculating Douglas' broth, rabbit's blood-agar plates, eosin-methylene blue agar plates, Fletcher's mediums, Löffler's mediums and deep meat tubes for incubation under aerobic and anaerobic conditions. Bacteriologic studies of most of the remaining 127 rats were limited to the inoculation of rabbit's blood-agar plates to detect the presence of salmonellas or contaminants and to the



Figures 1, 2, 3 and 4
(See legend on opposite page)

inoculation of Fletcher's mediums or sterile tap water to detect the presence of *L. icterohaemorrhagiae*.

The bacteriologic studies showed that extraneous contaminants were present rarely, *S. typhimurium* irregularly and *L. icterohaemorrhagiae* in 68 of the 139 rats (49 per cent) whose kidneys were investigated.

The results of the bacteriologic studies yielded no evidence to indicate that the presence of the inclusion bodies in the renal tissues was regularly associated with a single species of cultivable agent. It is of interest that renal inclusion bodies and *L. icterohaemorrhagiae* were demonstrated in the same percentage of rats (49 per cent). When attempts were made to relate these results for single animals, it became apparent that this finding represented a fortuitous coincidence, since some animals yielded leptospiras only, others inclusion bodies only, others both, and others neither. It was noted, however, that obviously old and senile rats not infrequently carried *L. icterohaemorrhagiae*, *S. typhimurium* and numerous inclusion bodies.

ATTEMPTS TO INDUCE RENAL INCLUSION DISEASE WITH TISSUE SUSPENSIONS

If a virus is the causative agent of renal inclusion disease of rats, tissue from kidneys known to contain numerous inclusion bodies might on transfer to susceptible hosts yield identical intranuclear alterations. In experiments designed to demonstrate a virus as the etiologic agent, suspensions of renal tissues from wild rats known to have the disease were injected into 33 albino rats.

The suspensions were prepared, as described, from the kidneys of wild rats shortly after removal. The kidneys utilized were limited to those which were known from microscopic examination of sections prepared by the frozen tissue technic to contain numerous inclusion bodies. The 33 albino rats received by the subcutaneous, or the intraperitoneal route 1 cc. of a 10 per cent suspension; 24 animals were given unfiltered tissue suspension, and 9 received Berkefeld-V filtrate. These animals were observed for from three to eighty-four days after injection.

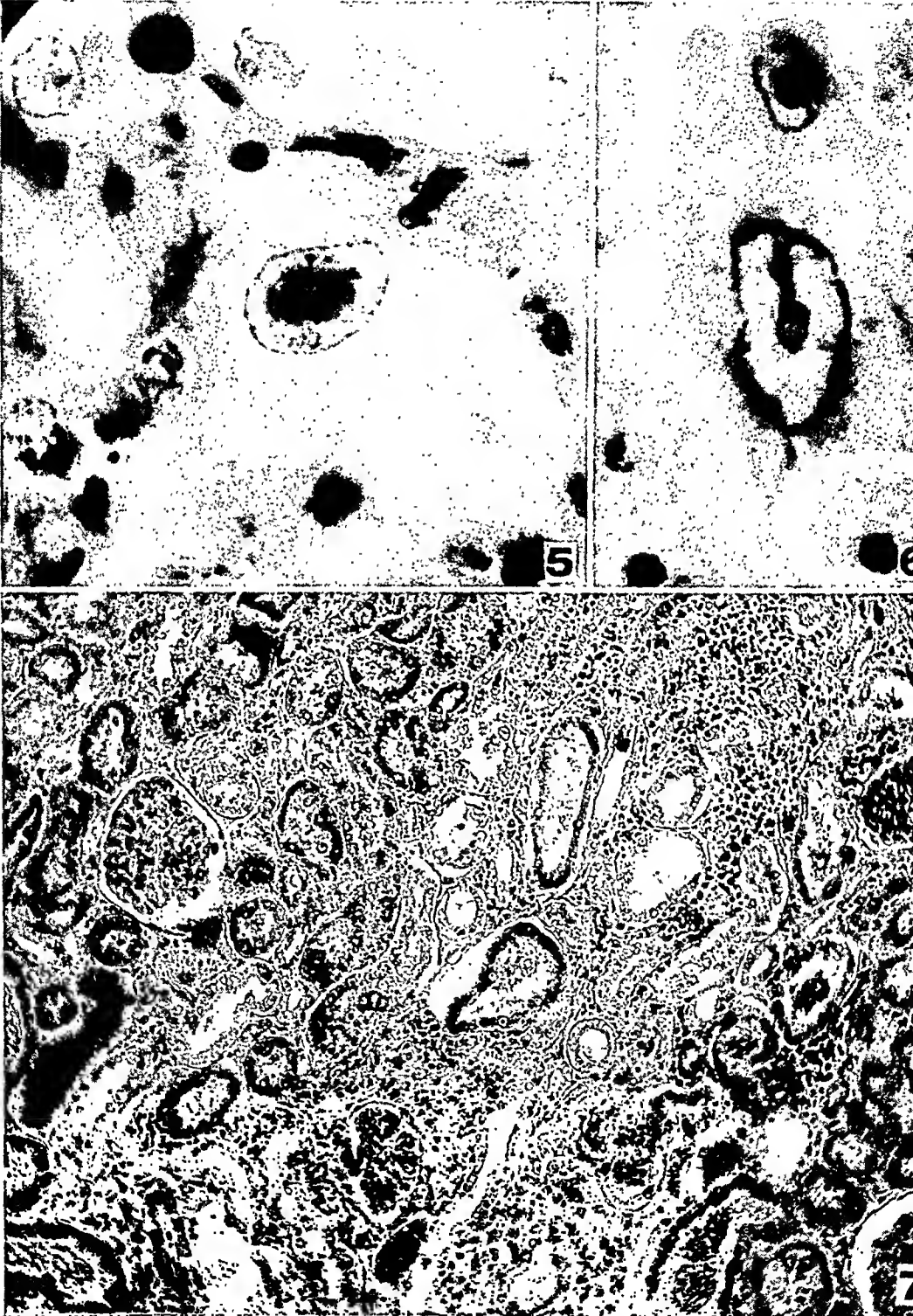
EXPLANATION OF FIGURES 1 TO 4

Fig. 1.—Section of kidney from wild rat 66. Each of 4 epithelial cells contains a single intranuclear inclusion body (arrows). Hematoxylin and eosin; $\times 1,500$.

Fig. 2.—Section of kidney from wild rat 187. Each of two greatly enlarged cells contains an intranuclear inclusion body. It can be seen that the basophilic chromatin is concentrated around the inclusion bodies and that the nucleoli are situated peripherally. Hematoxylin and eosin; $\times 1,500$.

Fig. 3.—Section of kidney from wild rat 175. The intranuclear inclusion bodies present in two cells (arrows) are markedly acidophilic and somewhat granular, resembling a type A intranuclear inclusion body.

Fig. 4.—Section of kidney from wild rat 82. A single, greatly enlarged cell and nucleus with its contained inclusion body (arrow) stands out in contrast to the normal cell. Hematoxylin and eosin; $\times 1,500$.



Figures 5, 6 and 7

(See legend on opposite page)

When none of the recipients showed evidence of clinical disease during the period of observation, and when microscopic examination of renal and salivary gland tissues from each rat failed to reveal intranuclear inclusion bodies, we concluded that a virus probably was not the causative agent of renal inclusion disease of wild rats.

ATTEMPTS TO PRODUCE INCLUSION BODIES BY INJECTING A CHEMICAL SUBSTANCE

When transmission experiments utilizing tissues known to contain inclusion bodies failed to yield clinical or histologic evidence of renal inclusion disease, resort was made to the experimental approach successfully employed by Olitsky and Harford³ for the induction of intranuclear inclusion bodies by injection of chemical substances. These investigators demonstrated intranuclear inclusion bodies in the phagocytic mononuclear and giant cell constituents of foreign body tissue reactions that followed subcutaneous injection of selected aluminum and ferric compounds and carbon. Since we had found the renal inclusion disease of rats to be largely confined to animals captured near sewers and dumps, it seemed possible that a toxic agent, e. g., a metal, might be ingested and absorbed from the gastrointestinal tract, to produce changes in renal epithelial cells during excretion. Accordingly, we selected aluminum hydroxide to be injected intravenously in animals believed to be susceptible.

Twenty-nine mature albino rats were selected as recipients. For control purposes, so as to rule out the possibility of preexisting renal inclusion bodies, a kidney was removed from each rat. Forty-five days later, after microscopic study had failed to reveal inclusion bodies in a single one of the kidneys from these normal animals, 0.2 to 0.5 cc. of aluminum hydroxide prepared according to the directions for making Willstätter's type C gel¹⁶ was injected into the tail vein of each of these rats. Fourteen animals died within seventy-two hours after receiving the injection. The data that relate to the 15 survivors are presented in table 3.

It can be seen in table 3 that 14 of the 15 survivors were given a second injection, but that 8 of these died within twenty-four hours. This

16. Willstätter and Kraut.¹¹ Sabin.¹²

EXPLANATION OF FIGURES 5 TO 7

Figs. 5 and 6.—Sections of kidney from wild rats 175 and 182. Both photographs show additional examples of the cellular alterations that occur in renal inclusion disease of wild rats. It can be seen that two of the cells are enlarged. Hematoxylin and eosin; $\times 1,500$.

Fig. 7.—This section from wild rat 66 is selected to show the type of histologic alteration present in many wild rat kidneys. Damaged glomeruli and tubules, round cell infiltration and fibrotic changes are present. Hematoxylin and eosin; $\times 150$.

made sections available for microscopic study from the remaining kidney and other tissues of 8 animals that survived a single injection for from thirteen to forty-nine days and from 7 that survived two injections whose deaths occurred from thirty-six to ninety-four days after the first injection, or from seven to eighty days after the second injection. It would seem that ample time had been allowed for tissue changes to develop. The results, however, were negative, for microscopic examination uniformly failed to show inclusion bodies.

COMMENT

The discovery of intranuclear inclusion bodies of Cowdry's type B in the renal cells of a rat captured in Rochester, N. Y., led us to sample the rat population in Rochester and other cities of the United States to learn whether this type of change was common among rats. Accordingly, 139 wild rats were procured from Rochester and such widely separated cities as Detroit, Denver and San Francisco. Inclusion bodies were found in the epithelial cells of 49 per cent. The incidence was apparently

TABLE 3.—*Data That Relate to the Attempt to Induce Intranuclear Inclusion Bodies in the Renal Epithelial Cells of Albino Rats by One or Two Intravenous Injections of Aluminum Hydroxide*

Rat	Aluminum Hydroxide Injected Intravenously, Cc.		Time Interval	
	Dose 1	Dose 2	Days to Dose 2	Days to Death
A 1.....	0.5	0.3	13	13
A 2.....	0.5	0.2	18	18
A 3.....	0.2	...	0	23
A 4.....	0.2	0.3	27	27
A 5.....	0.25	0.3	27	27
A 6.....	0.3	0.5	27	27
A 7.....	0.5	0.3	27	27
A 8.....	0.5	0.25	27	36
A 9.....	0.4	0.1	49	59
A 10.....	0.5	0.4	49	49
A 11.....	0.5	0.4	49	49
A 12.....	0.2	0.3	21	70
A 13.....	0.25	0.3	27	76
A 14.....	0.5	0.3	14	94
A 15.....	0.3	0.3	14	94

unrelated to geographic origin, but was highest among old urban rats. On the other hand, inclusion bodies were not found in renal tissues from immature rats, from rats captured in rural areas, or from albino rats, irrespective of their age.

This demonstration of intranuclear inclusion bodies in about half of the wild rats led us to suspect that we might be dealing with a disease caused by a virus, for the presence of inclusion bodies in cells is often

evidence of virus activity. For one type of intranuclear inclusion body (Cowdry's type A) this association is so regular that the microscopic demonstration of type A intranuclear inclusion bodies in tissue cells is commonly accepted as pathognomonic of virus infection. On the other hand, the presence of intranuclear inclusion bodies of Cowdry's type B is but suggestive of virus activity. As the intranuclear inclusion body in renal inclusion disease of rats is of the latter variety, it seemed pertinent that our studies should be directed toward learning whether this malady could be explained by recovery of a virus; if not, toward securing data on which to appraise the significance of the inclusion bodies. Accordingly, our experiments were designed to enable us (1) to isolate a virus and (2) to reproduce the intranuclear alteration by inoculating the inclusion-laden tissues into normal animals and by utilizing aluminum hydroxide, a chemical known to produce inclusion bodies.

Our experiments yielded no evidence that a virus is the etiologic agent of the renal inclusion disease of rats or that the cellular alterations of this disease can be reproduced by the injection of aluminum hydroxide. We can, therefore, but conjecture as to the nature and the mode of action of the agent responsible for the pathologic picture. It was noted that the disease was limited to urban rats and that the incidence of occurrence and the number of inclusion bodies increased with age. Moreover, rats captured in refuse dumps or downtown showed more evidence of the disease than suburban rats. This suggests that repeated ingestion of some toxic agent, perhaps a heavy metal, might be responsible. Another possibility is that the intranuclear inclusion bodies reflect the residuum of an inapparent virus infection of slight pathogenicity and long duration. The demonstration in this same group of rats of inapparent infections with *L. icterohaemorrhagiae* and *S. typhimurium* and the capacity of rats to harbor bacteria,¹⁷ rickettsias¹⁸ and viruses¹⁹ as inapparent infections possibly lend some support to this idea.

It is of interest that renal inclusion bodies and recovery of *L. icterohaemorrhagiae* were demonstrated for the same percentage of rats (49 per cent). However, attempts to relate these findings for single animals made it apparent that this observation represented a fortuitous coincidence, since some animals yielded leptospiras only, others inclusion bodies only, others both, and others neither leptospiras nor inclusion bodies. It was noted, however, that obviously old and senile rats not only showed evidence for extensive inclusion body disease but also carried *L. icterohaemorrhagiae* and *S. typhimurium*.

17. Hülphers, G., and Henricson, T.: *Svensk vet. tidskr.* **48**:197 and 245, 1943; abstracted, *Biol. Abstr.* **20**:383, 1946.

18. Dyer, R. E.: *Am. J. Trop. Med.* **21**:163, 1941.

19. Burnet, F. M.: *J. Path. & Bact.* **42**:213, 1936.

SUMMARY

The kidneys from 139 wild rats (*Rattus norvegicus*) captured in widely separated parts of the United States were examined microscopically for the presence of intranuclear inclusion bodies. When it was found that Cowdry's type B intranuclear inclusion bodies were present in the epithelial cells of the renal tubules of 68 rats (49 per cent), two groups of experiments were carried out to learn whether the causal agent was infectious in nature or a chemical.

Albino rats of the Wistar strain were used as experimental animals. To rule out preexisting inclusion bodies in the kidneys, one kidney from each albino rat was removed for microscopic examination forty-five days before use of the rat.

The first group of experiments employed fresh renal tissues that were known from examination of sections prepared by the frozen section technic to contain numerous inclusion bodies. Filtered and unfiltered suspensions of these tissues were injected into 33 normal albino rats by subcutaneous and intraperitoneal routes. When animals were killed in from three to eighty-four days after injection, none showed any inclusion bodies.

In the second series of experiments, Willstätter's type C alumina gel (0.2 to 0.5 cc. in one or two doses) was injected intravenously into 29 rats. Of these, 15 survived and died or were killed in from thirteen to ninety-four days after injection. Again, in none of the rats were intranuclear inclusion bodies found.

The photographs were made by Mr. Mervyn C. Orser.

PULMONARY ADENOMATOSIS RESEMBLING JAGZIEKTE IN THE GUINEA PIG

. ROBERT F. NORRIS, M.D.

PHILADELPHIA

PULMONARY adenomatous lesions of the guinea pig were described by Sternberg¹ in 1904 and Spronck² in 1907. In 1926 Grumbach³ induced adenomatous lesions in the lungs of guinea pigs by inoculating a diphtheroid bacillus isolated from the lymph nodes of a patient with Hodgkin's disease. Grumbach was impressed by the fact that these lesions were similar to those of jagziekte of sheep. Cowdry and Marsh,⁴ who also examined the slides, agreed that the experimentally induced lesions resembled those of jagziekte as well as those of the progressive pneumonia occurring in the sheep of Montana.

The present report is concerned with a guinea pig which had adenomatous lesions of one lung, also resembling jagziekte of sheep. Although the guinea pig was inoculated prior to death with pleural fluid from a patient suspected of having pulmonary tuberculosis, it will be seen from the following clinical history that the inoculation probably was not responsible for the lesions.

REPORT OF CASE

W. R. B., a male Negro aged 18, was admitted to the Hospital of the University of Pennsylvania, May 7, 1946, complaining of pain in the left side of the chest. Following the onset of a cold several days before admission, he felt feverish and began to cough up reddish sputum. On admission the diagnosis was lobar pneumonia of the lower lobe of the left lung, which was confirmed by a roentgenogram of the chest. The clinical signs and symptoms disappeared promptly after the patient was treated with sulfadiazine and penicillin, and he was discharged from the hospital on May 15, eight days after admission.

He was readmitted to the hospital on May 24 because of a recurrence of pain in the left side of the chest. At this time a large collection of fluid was present in the left pleural cavity. He was treated by repeated aspirations. Before the pleural effusion had entirely disappeared, he signed his release from

From the William Pepper Laboratory of Clinical Medicine, University of Pennsylvania.

1. Sternberg: *Verhandl. d. deutsch. path. Gesellsch.* **6**:134, 1904.
2. Spronck, C. H. H.: *Nederl. tijdschr. v. geneesk.* **43**:1033, 1907.
3. Grumbach, A.: *Bull. Assoc. franç. p. l'étude du cancer* **15**:213, 1926.
4. Cowdry, E. V., and Marsh, H.: *J. Exper. Med.* **45**:571, 1927.

the hospital on June 8 and was not seen again in the dispensary until Jan. 4, 1947, when he was suffering from acute gonorrheal urethritis. At this time in a roentgenologic examination of the chest the heart and lungs were normal, but the left side of the diaphragm fluoroscopically was slightly flattened and the left costophrenic sulcus was blunt. Those abnormalities were the only sequelae of the previous pneumonia and pleural effusion.

During the second admission, several specimens of aspirated pleural fluid were uniformly clear and straw-colored and were sterile when cultured by routine bacteriologic methods. One specimen cultured for tubercle bacilli likewise showed no growth. Two guinea pigs were inoculated with this specimen. Both animals

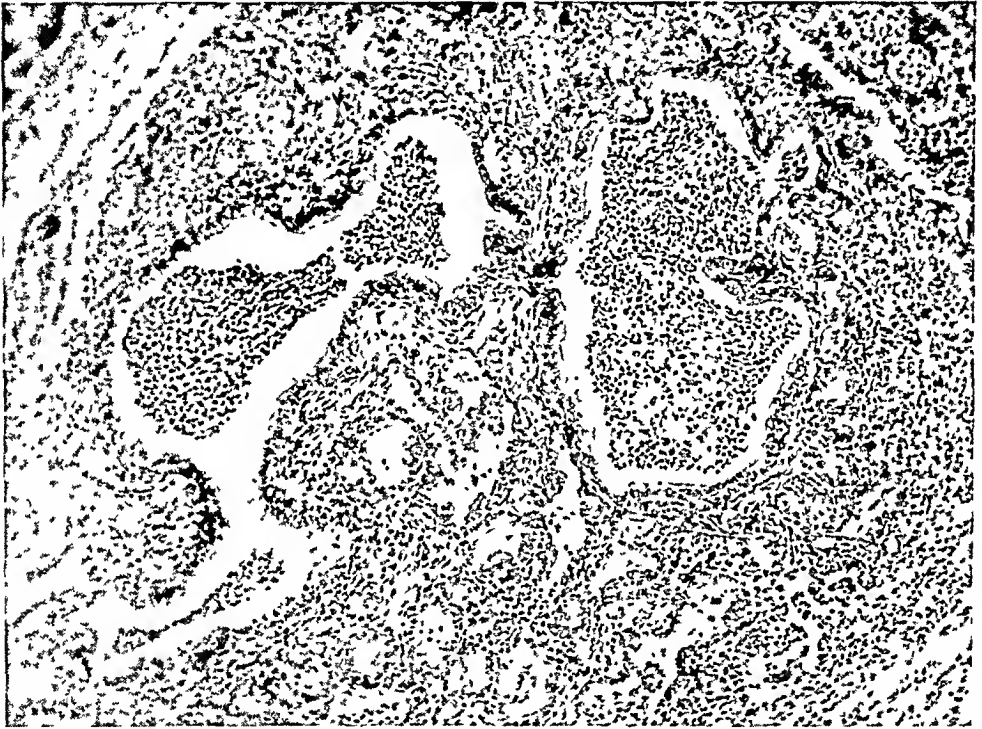


Fig. 1.—There are papillary projections of columnar epithelium in two widely dilated small bronchi. The lumens contain many polymorphonuclear neutrophils and large mononuclear and polymorphonuclear phagocytes. The neighboring alveoli are lined by epithelial cells of like character. Adenomatous proliferation of the epithelium is extending centrifugally, and the uninvolved alveoli appear compressed.

were alive and appeared well when killed two months after inoculation. The organs of one guinea pig were grossly and microscopically normal. The lesions found in the other guinea pig will be described in detail.

Necropsy of the Guinea Pig.—The technician who killed the animal did not observe any gross lesions at the necropsy. It was not until the microscopic sections were prepared that the following abnormalities were discovered.

In one lung there were discrete and confluent foci of adenomatous epithelial proliferation. In roughly circular zones about the bronchi the neighboring alveoli

were completely lined by epithelial cells of the same character. An adenomatous appearance thus resulted. Peripherally the lesions were not encapsulated, but the nearby alveolar stroma was condensed. The epithelial proliferation was sharply margined, but throughout the circumference of the lesions epithelial cells were proliferating into previously normal alveoli but had not yet completely lined them. The parenchyma of the lung between lesions showed no abnormalities other than atelectasis, which was possibly due in part to compression (fig. 1).

The epithelial cells comprising the lesions were identical in both the bronchi and the alveoli. They were larger and more swollen in appearance than those of normal bronchi, and none were ciliated. Although in most areas the bronchi and alveoli were lined by a single layer of epithelial cells, in places the cells were heaped up to form papillary folds which projected into the lumens. Most of these papillae had cores of fibrous tissue. Individually the cells were generally of uniform size and showed little pleomorphism. The nuclei were usually oval, vesicular and moderately hyperchromatic. Small basophilic nucleoli were often present. Mitoses were not seen. Most often the nuclei were basilar. The cytoplasm was slightly granular but translucent and practically colorless (fig. 2A).

Within the cytoplasm of many cells were faintly stained oval bodies, approximating the size of nuclei, but often larger, which were more opaque than the surrounding cytoplasm and which appeared to contain minute darkly staining granules. The individual granules were surrounded by narrow translucent zones. Stained with Giemsa or phloxine and methylene blue, however, these granules were less distinct than with hematoxylin and eosin. The bodies containing these granules were sharply margined, but distinct enclosing membranes were not distinguished. Most of these bodies were situated between the nucleus and the free margin of the cell. At times they were free in the lumens. Some of these were engulfed by large mononuclear or polymorphonuclear cells resembling phagocytes (fig. 2B).

Within the lesions the alveolar septums were thickened by increased amounts of collagenous and reticular tissue and by small numbers of extravasated plasma cells, mononuclear phagocytes and polymorphonuclear neutrophils. Only a few large foci of lymphoid hyperplasia were seen in the vicinity of bronchi, and there was no scarring or proliferation of myxomatous fibrous tissue.

Within the lumens of the bronchi were large numbers of polymorphonuclear neutrophils and a few large mononuclear phagocytes. Large mononuclear phagocytes, some of which were multinuclear, were also present in the lumens of the alveoli, but in only a few were polymorphonuclear neutrophils present. Gram and Giemsa stains did not demonstrate bacteria. Unfortunately when the lesions were discovered there was no unfixed tissue from which further bacteriologic studies could be made.

In a section from the opposite lung there were no large adenomatous lesions, but there was reduplication of the epithelium in several of the small bronchi. Extending into the alveoli adjacent to these bronchi were small areas of epithelial proliferation resembling those in the other lung. Inflammatory exudate and lymphoid hyperplasia were not present in this lung. Except for compensatory emphysema, this lung showed no other lesions.

In a section of the spleen, small zones of fibrosis, not yet hyaline, encircled many of the malpighian bodies, but no other lesions were seen. Nothing abnormal was seen in sections of the liver, a kidney and inguinal lymph nodes.

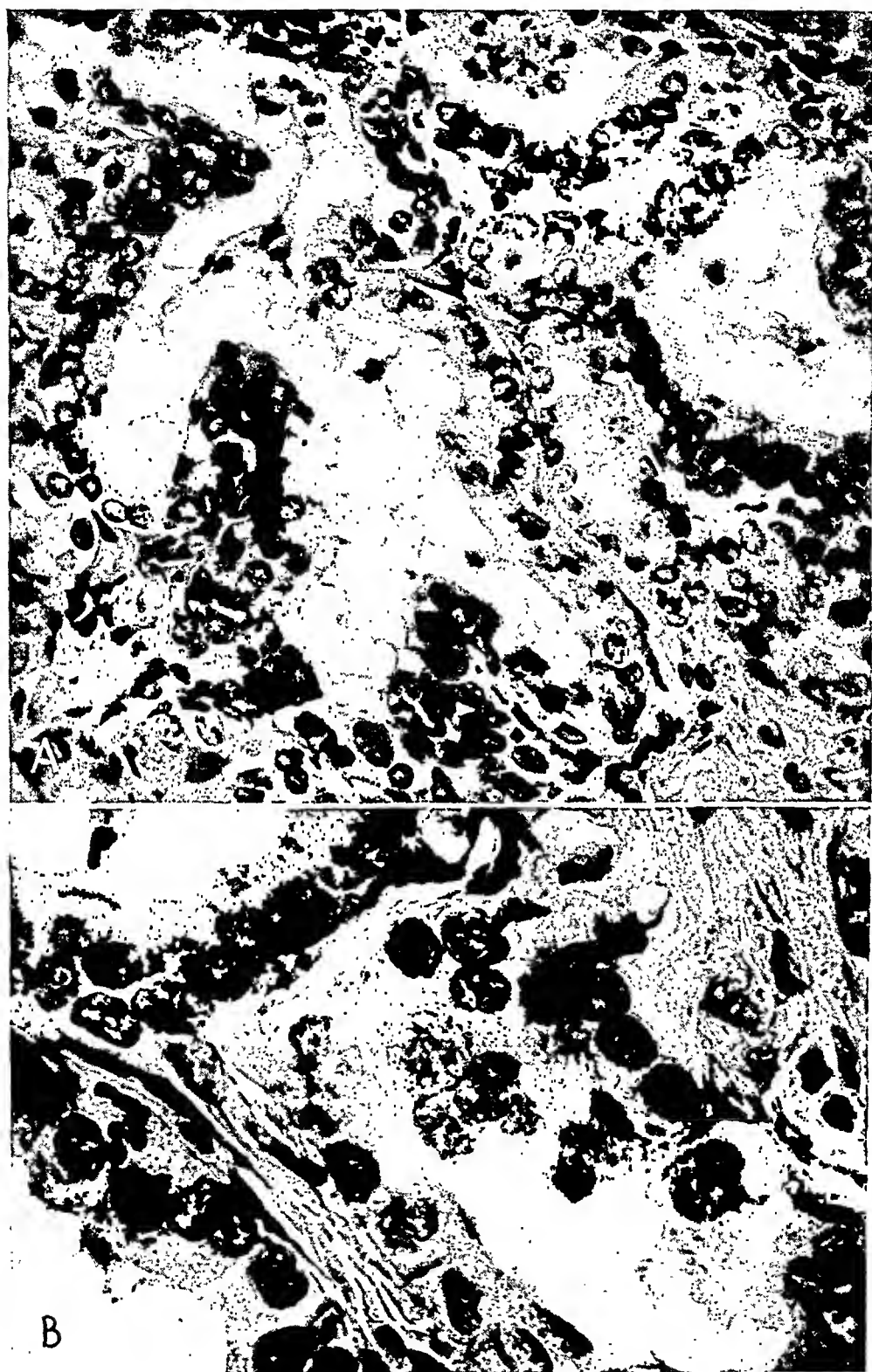


Figure 2

(See legend on opposite page)

COMMENT

The pulmonary adenomatous lesions described are thought to have been essentially benign in that invasion of the alveoli occurred without apparent destruction of the septums. Also the individual epithelial cells were well differentiated and were generally uniform in contour. However, blocks were not available from a sufficient number of organs to exclude the presence of metastasis. Since the epithelium of the smaller bronchi corresponded with that lining the alveoli, it may be assumed that epithelial proliferation began in the bronchi and extended peripherally to involve the neighboring alveoli.

So far as can be determined from the descriptions, the lesions of the present case were similar to those in guinea pigs described by Sternberg¹ and later by Spronck,² and to those induced experimentally by Grumbach.³ Sternberg likewise believed that the epithelial proliferation began in the smaller bronchi.

Since the lesions in the present case also were thought to resemble the jagziekte occurring in sheep, at the suggestion of Dr. Baldwin Lucké slides were submitted to Major W. A. Bennett, of the Army Institute of Pathology, Washington, D. C., and to Dr. C. L. Davis, of the Bureau of Animal Industry, Denver, and they expressed the following opinions. Major Bennett expressed the belief that the lesions were compatible with jagziekte but pointed out that it is unusual for the disease to begin in the smaller bronchi. Dr. Davis, however, expressed doubt as to whether a diagnosis of jagziekte was justified, especially since the disease is primarily one of the ovine species. Both emphasized the likelihood that the epithelial hyperplasia was secondary to chronic inflammation.

In a consideration of the arguments for and against a relationship with jagziekte in the present case, it may be said that the lesions resembled those in sheep in that the epithelial proliferation did not appear to be cancerous and the individual epithelial cells were large nonciliated columnar cells with clear cytoplasm. The papillary projections were also similar to those of jagziekte and the large oval or circular granular bodies seen in the cytoplasm of the cells and in the lumens of the alveoli resembled those observed by Dungal⁵ in the sheep of Iceland. The observation that the epithelial proliferation apparently originated in the small bronchi may be considered as one which would differentiate the condition from jagziekte, because Cowdry⁶ reported that the lesions

5. Dungal, N.: Proc. Roy. Soc. Med. **31**:497, 1938; Am. J. Path. **22**:737, 1946.

6. Cowdry, E. V.: J. Exper. Med. **42**:323 and 335, 1925.

Fig. 2.—With greater magnification (*A*) papillary projections of translucent columnar alveolar epithelium are apparent. The epithelial cells show little pleomorphism. In many cells, between the nucleus and the free margin are opaque spherical or oval bodies containing darkly staining granules. In *B*, with still greater magnification (oil immersion objective), several large spherical bodies containing darkly staining granules may be seen lying free in the lumen of the alveolus.

of the latter begin in the alveoli and not in the bronchi. Mitchell,⁷ de Kock,⁸ and Dungal⁹ stated, however, that the lesions may originate in the smaller bronchi. There was also absence of marked lymphoid hyperplasia, described by Mitchell⁷ as common in jagziekte. But de Kock⁸ was unable to confirm this characteristic and raised the question whether Mitchell might have been dealing with two separate diseases. In the present case, the lack of proliferation of myxomatous and fibrous stroma is not necessarily an argument against the diagnosis, since these changes usually occur late in the course of the disease and are not always present.

It is uncertain whether the inflammatory exudate present especially in the bronchi represented antecedent bronchitis which initiated the proliferation of epithelium or secondary infection. The fact that acute inflammatory exudate was more abundant in the bronchi than in the alveoli and that inflammatory exudate was lacking in the opposite lung favors the latter hypothesis, which, if true, is in agreement with the observation⁹ that secondarily acquired pneumonia is not uncommon in jagziekte.

From the evidence presented it may be concluded that the adenomatous lesions of the present case are morphologically similar to jagziekte of sheep. Except for this resemblance, there is no evidence that the lesions are etiologically identical.

Although the cause of jagzeikte is still unknown, most observers believe that the disease is infectious.⁵ Attempts to transmit the disease from sheep to other animals, including guinea pigs, have not been successful. Nevertheless, the fact that pulmonary adenomatosis occurs in guinea pigs indicates that if jagziekte is ultimately proved to be an infection it may yet be transmitted to guinea pigs under proper conditions of experiment.

Since the patient whose pleural fluid was injected into the guinea pig recovered from the lobar pneumonia and does not at present show any roentgenologic evidence of pulmonary disease, it is unlikely that the inoculation was significant in the causation of the pulmonary adenomatosis of the guinea pig.

SUMMARY

Pulmonary adenomatosis involving one lung of a guinea pig is described. The lesions resemble those of jagziekte occurring in sheep. Although the guinea pig was originally inoculated with the pleural fluid of a patient convalescing from lobar pneumonia, there is no evidence that the patient suffered from a similar disease.

7. Mitchell, D. T., in Third and Fourth Annual Reports of the Director of Veterinary Service, Union of South Africa, 1915, p. 585.

8. de Kock, G., in Fifteenth Annual Report of the Director of Veterinary Service, Union of South Africa, 1929, p. 611.

9. Dungal.⁵ de Kock.⁸

STRUCTURAL CHANGES IN THE KIDNEYS OF RATS WITH EXPERIMENTAL CHRONIC HYPERTENSION

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IN A PREVIOUS communication studies of the structural changes observed in the visceral blood vessels, the heart and the kidneys of rats with experimental chronic hypertension were reported.¹ It was stated that the blood vessels revealed minimal or no microscopic changes and that the left cardiac ventricle showed hypertrophy with its concomitant changes. The latter changes were usually commensurate with the degree and the duration of the observed elevation of blood pressure. Renal lesions apparently responsible for the hypertension were observed in every kidney to which a ligature was applied. Focal changes attributed to the hypertension were observed in the opposite, unligated kidney.

In this paper, studies are reported on an additional series of rats in which experimental chronic hypertension had been induced by various methods. Particular attention is paid to the renal lesions producing the hypertension and to the renal lesions resulting from the hypertension.

METHOD

Twenty piebald rats of the Evans-McCollum strain were used. In 13 of these a figure of eight ligature was applied to the right kidney as previously described, the left kidney being left intact.² Within ten to sixteen weeks chronic hypertension developed in 9 of these rats. Of the remaining 4 rats, in which the procedure did not result in hypertension, 3 had a ligature applied then to the left kidney, and in 1 the left kidney was removed. In 2 rats chronic hypertension was induced by removing the right kidney without interfering with the left kidney. As controls, the left kidneys of 5 rats in which ligation of the right kidney failed to produce hypertension were used.

This study was aided by a grant from the John and Mary Markle Foundation.

From the Department of Pathology, University of Oklahoma School of Medicine, and the Department of Experimental Medicine, Southwestern Medical College.

1. Halpert, B., and Grollman, A.: *Proc. Soc. Exper. Biol. & Med.* **62**:273, 1946.

2. Grollman, A.: *Proc. Soc. Exper. Biol. & Med.* **57**:102, 1944.

Blood pressure determinations were made on the unanesthetized animals by the plethysmographic method of Williams, Harrison and Grollman³ Blood pressure recordings were made throughout the period of observation. All the animals used had chronic elevation of blood pressure for ten weeks or longer and appeared to be free of infection. The rats were killed after their blood pressure had been maintained at mean levels between 160 and 200 mm. of mercury for periods varying from ten to twenty weeks. The pertinent data concerning the mode of induction, the mean level of blood pressure and the duration of hypertension are recorded in the accompanying table.

Mode of Induction, Mean Level and Duration of Hypertension

Rat	Condition of Kidneys		Mean Blood Pressure, Mm of Mercury	Duration of Hypertension, Weeks
	Right	Left		
43	Ligated	Intact	160	10
44	Ligated	Intact	180	12
45	Ligated	Intact	190	14
46	Ligated	Intact	200	16
47	Ligated	Intact	160	12
49	Ligated	Intact	180	13
50	Ligated	Intact	190	15
51	Ligated	Intact	170	14
52	Ligated	Intact	180	15
38	Ligated	Ligated	170	15
39	Ligated	Ligated	180	16
41	Ligated	Ligated	200	18
40	Ligated	Ablated	180	12
31	Ablated	Intact	200	20
48	Ablated	Intact	180	14

Microscopic studies were made of organs fixed in solution of formaldehyde U.S.P. diluted 1:25 embedded in paraffin and stained with hematoxylin and eosin. The kidneys and the hearts of the 15 rats with chronic hypertension were studied, and in 5 animals the lungs, the spleen and the liver and occasionally other organs (pancreas, intestine, mesentery, adrenal, brain) were also examined.

OBSERVATIONS

The hearts of all of the 15 rats with hypertension disclosed varying degrees of hypertrophy of the left ventricle. In fact, the hypertrophic changes as objective criteria paralleled the degree and the duration of the hypertension. The increase and the variation in size of the myocardial fibers are well illustrated in figure 1A from rat 40 in which the hypertension was induced by a figure of eight ligation of the right kidney and subsequent operative removal of the left kidney. The mean blood pressure at the end of twelve weeks was 180 mm. of mercury. In figure 1B are shown some of the changes occurring in the right kidney.

3. Williams, J. R., Jr ; Harrison, T. R., and Grollman, A.: J. Clin Investigation 18:373, 1939.

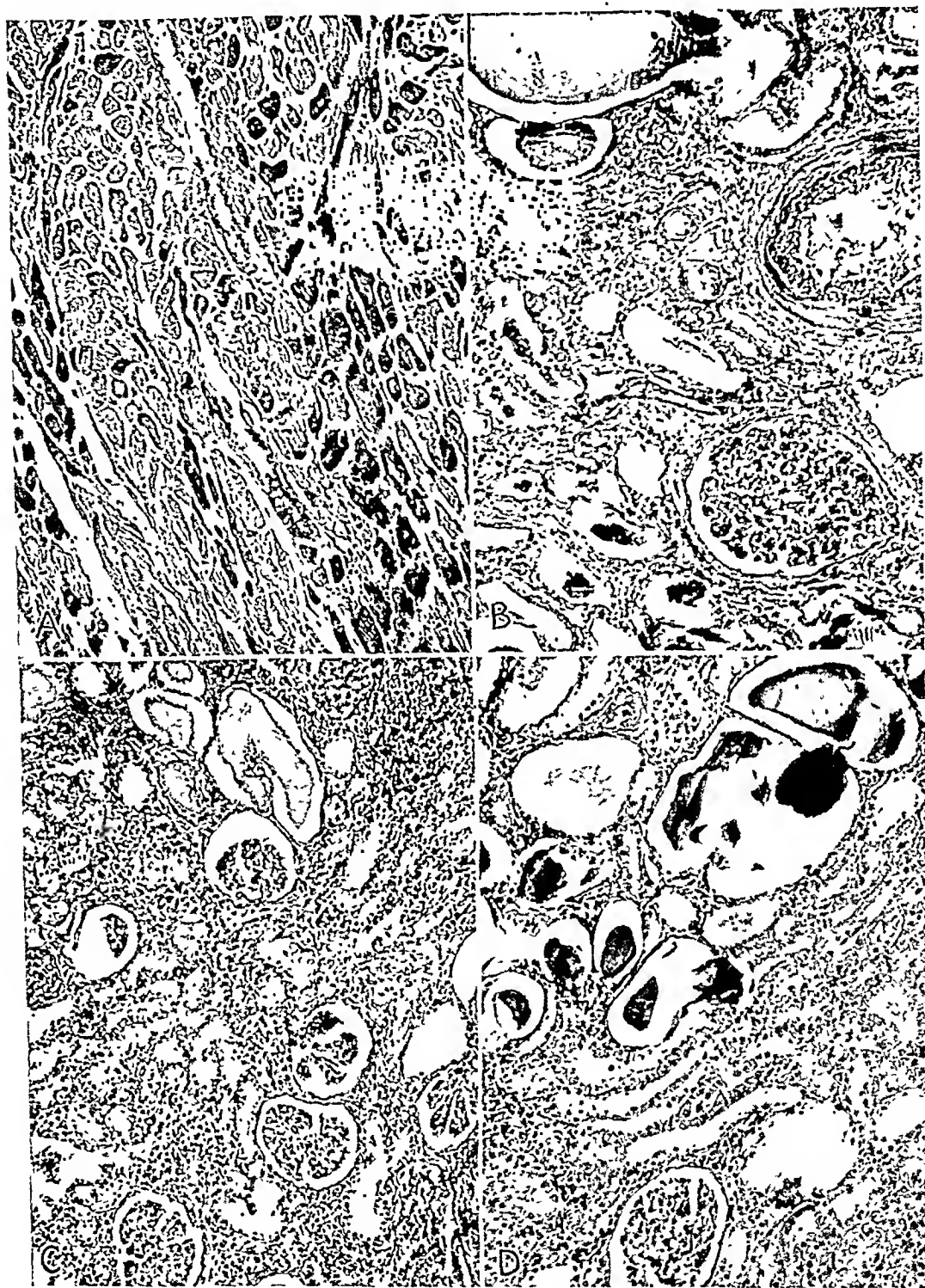


Fig. 1.—*A*, changes in the myocardium of the left ventricle and *B*, changes in the right kidney of rat 40, in which hypertension was induced by a figure of eight ligation of the right kidney and subsequent operative removal of the left kidney. $\times 100$. *C* and *D*, comparable changes in the right and left kidneys of rat 44, in which hypertension was induced by a figure of eight ligation of the right kidney, the left being left unmolested. The cellular reaction in the right kidney is toward the ligature. $\times 100$.

In this series as in the series of rats previously reported, the blood vessels of all of the organs examined revealed minimal or no changes.

In the 9 rats (table) in which a figure of eight ligature was applied to the right kidney, the left being left intact, the changes observed in both kidneys were essentially the same as those previously reported. The right kidneys, to which the ligatures were applied, were decreased in size, with deep fissures subdividing the surface. On the cut surfaces the widths of the cortical and medullary zones were decreased in proportion to the damage of the renal parenchyma. The cotton thread with which the kidneys had been constricted was demarcated microscopically by a zone of connective tissue infiltrated with lymphocytes, plasma cells and large mononuclear cells. The last often contained brown granules. Occasional giant cells of the foreign body type were also seen. The nearby renal parenchyma was transformed into scar tissue. The changes in the glomeruli ranged from a slight focal fusing of the capillaries with Bowman's capsule to complete disappearance of the capsular space and fibrous connective tissue replacement of the glomerulus with varying degrees of hyaline change. The convoluted and collecting tubules of such glomeruli were completely obliterated or were distended with a bright pink homogeneous material (figs. 1C and 2A).

The damage of the renal parenchyma of all of these kidneys was marked and was estimated to involve over half of the nephrons. The degree of involvement varied but was usually most marked about or near the course of the constricting thread. Occasional islands of fairly intact renal parenchyma were seen between the injured nephrons or their remains. In none were there any obvious changes in the large or smaller intrarenal blood vessels.

The left unmolested kidneys of these 9 rats were not distorted grossly. The microscopic changes were focal and involved an occasional single glomerulus or groups of glomeruli. They consisted of varying degrees of obliteration of the glomerular pattern. The convoluted and collecting tubules had coarse convolutions, contained a homogeneous bright pink material and were lined by flat cells. They occupied spaces at times two or more times the diameter of an intact glomerulus (figs. 1D and 2B). Here, too, no obvious changes were seen in the blood vessels.

A comparison of the changes in the right ligated and the left unmolested kidneys of this group of rats (figs. 1C and D and 2A and B) suggested that the two kinds of changes, one inducing the hypertension and the other produced by the hypertension, could be identified with reasonable certainty. The distorting coarse atrophy and scarring reducing the renal parenchyma along the constricting thread applied to the right kidney obviously were the lesions inducing the hypertension. The changes present in all of the left kidneys must be assumed to be

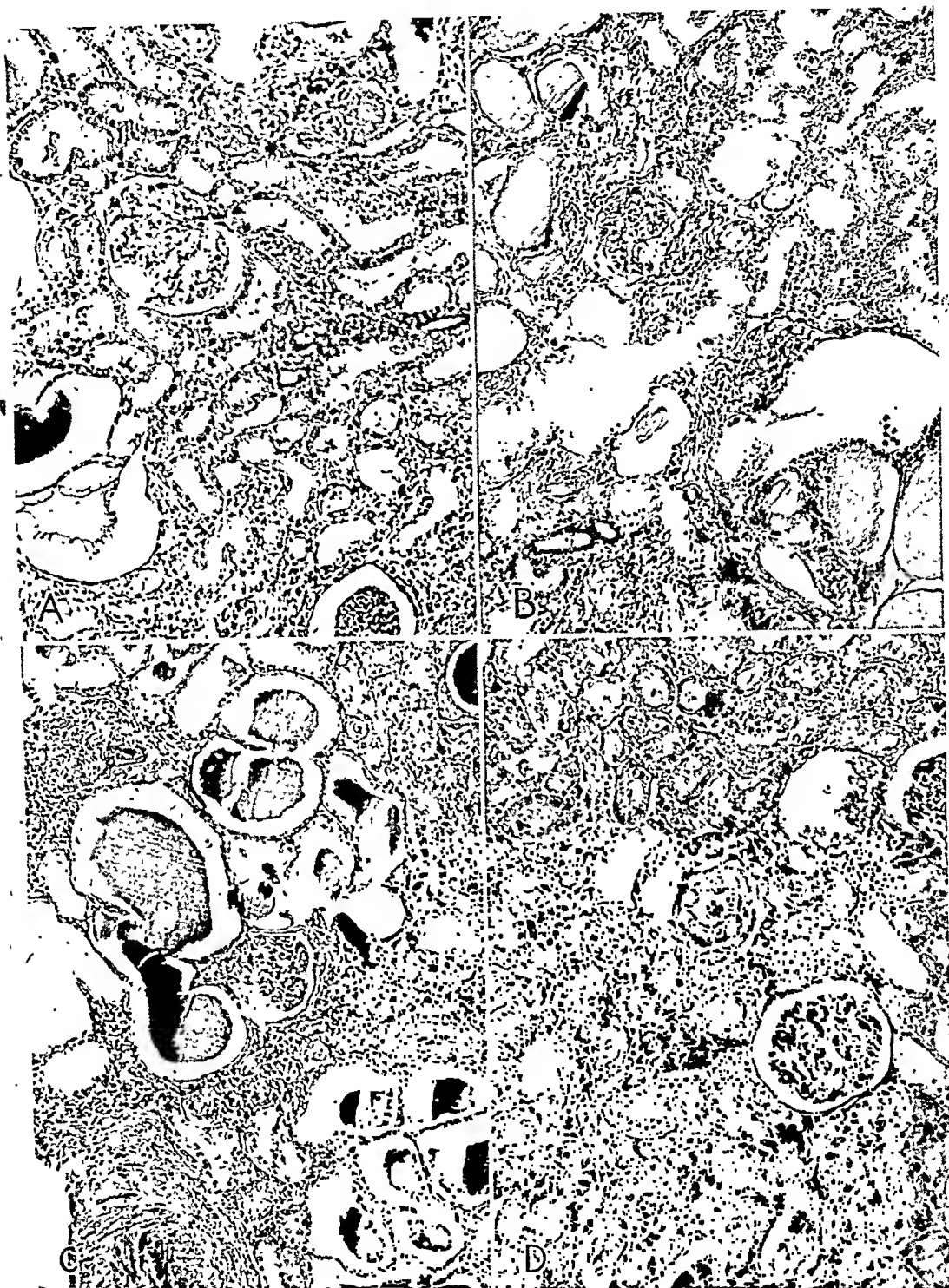


Fig. 2.—*A*, changes in the right and, *B*, changes in the left kidney of rat 46, in which the right kidney had been ligated, the left being unligated. The changes in the left kidney, *B*, are assumed to be due to the hypertension. $\times 100$. *C*, changes in the right and, *D*, changes in the left kidney of rat 41 in which a ligature was applied to the right kidney and subsequently one also to the left kidney. The changes in the right kidney are marked. The field chosen in the left kidney is relatively free of involvement. $\times 100$.

due to the hypertension. The comparable changes observed in the right kidneys are also attributable to the hypertension.

The mechanism by which the hypertension injures the glomeruli is not clear. In both kidneys, however, the lesions attributed to the hypertension involved primarily the glomeruli themselves and then the tubules belonging to them. With the exception of the glomerular capillaries no changes were seen in any of the intrarenal blood vessels. Consequently it must be assumed that the changes in the nephrons were accomplished by injury of the intraglomerular capillaries.

In the 3 rats (table) in which a figure of eight ligature was applied to the right kidney without producing hypertension and three months later one was applied to the left kidney, changes were observed in both kidneys. The scarring and atrophy in both kidneys were similar to those seen in the right kidneys of the previous group. The changes in the right kidneys, however, seemed older than those in the left and were less extensive, leaving more uninvolved renal substance (fig. 2C and D). Changes produced by the hypertension as seen in the left unmolested kidneys of the first group were also seen in the right kidneys of these 3 rats.

In the rat in which the left kidney was removed subsequent to ligation of the right kidney the involvement of the right kidney was particularly extensive (fig. 1B). In this kidney there were few intact nephrons. Other nephrons were in all stages of injury: from recent obliteration of the glomerular pattern, where a pink-stained coagulum occupied the space inside Bowman's capsule with only ghosts of the capillaries remaining, to complete hyaline change of the glomeruli.

In the 2 rats in which hypertension was produced by removal of the right kidneys, the left kidneys were grossly not distorted. The microscopic changes were focal rather than diffuse and were quite similar to those seen in the left kidneys of the rats in which hypertension was induced by ligature of the right kidneys. Here, too, there were no obvious changes in the blood vessels other than those in the glomerular capillaries.

In the 5 control kidneys no gross changes were seen externally or on their cut surfaces. Microscopic examination disclosed good-sized glomeruli with intact convoluted and collecting tubules. No changes were seen in the stroma and blood vessels.

COMMENT

All the animals used in these experiments appeared to be free of infection. Animals manifesting acute illness or harboring suppurative lesions were considered unsuitable for the study of the effects of uncomplicated chronic hypertension and therefore were eliminated, since their use has led to unwarranted conclusions.

The hypertension in our experimental animals was apparently caused by damage or elimination of the renal parenchyma which reduced the number of available functioning nephrons. Such reduction was initiated by a figure of eight ligation of one or both kidneys or by ablation of one kidney without interference with the other. When chronic hypertension was once established, it caused further injury of the remaining nephrons whether one or both kidneys were present.

The induction of hypertension by unilateral nephrectomy, the opposite kidney not being interfered with, speaks against the assumption that a pressor agent liberated into the circulation from injured renal tissue is responsible for the development of the observed hypertension.⁴

The results of the present study support the view that neither damage of renal tissue nor ischemia nor intrarenal vascular changes are primary prerequisites for the induction of hypertension. It would rather appear that reduction of the total number of functioning nephrons is responsible for the development of the disorder. Hypertension once established damages additional nephrons, which further increases the hypertension. Therefore in the rat as in man it is a progressive disease the advance of which may be retarded but not abolished.⁵

SUMMARY

Experimental chronic hypertension was produced in 15 rats by damage or elimination of renal parenchyma which reduced the number of available functioning nephrons. Such reduction was initiated when a figure of eight ligature was applied to one or both kidneys or when one kidney was removed without interference with the other. The chronic hypertension thus produced resulted in hypertrophy of the left cardiac ventricle which paralleled the degree and the duration of the hypertension.

The data presented support the view that neither damage of renal tissue nor ischemia nor intrarenal vascular changes are primary prerequisites for the induction of hypertension but that it is the reduction of the total number of functioning nephrons which initiates the disorder.

Hypertension once established caused further injury to the remaining nephrons whether one or both kidneys were present. Therefore, chronic hypertension of the rat is a progressive disease, the advance of which may be retarded but not abolished.

4. Grollman, A.; Harrison, T. R., and Williams, J. R., Jr.: *Am. J. Physiol.* **139**:293, 1943. Loomis, D.: *Arch. Path.* **41**:231, 1946. Grollman, A., in Pincus, G.: *Recent Progress in Hormone Research: Proceedings of the Laurentian Hormone Conference*, New York, Academic Press, Inc., 1947, vol. 1, p. 371.

5. Grollman, A., in Goldring, W., and others: *Experimental Hypertension*, Special Publications, New York Academy of Science, 1946, vol. 3, p. 99.

THE FURTHER EFFECT OF THE LEUKOCYTOSIS-PROMOTING FACTOR OF EXUDATES WHEN INJECTED IN CONNECTION WITH INFLAMMATION

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IN STUDIES previously reported there was demonstrated in exudative material the presence of a factor capable of reasonably explaining the leukocytosis frequently accompanying inflammatory processes.¹ This factor when injected into animals not only induces a discharge of immature granulocytes of the bone marrow but produces a hyperplasia or growth of granulocytes and of megakaryocytes in the marrow.² Its activity in human beings suggests that the material may have a clinical application.³ It is well known that the prognosis of many infectious processes is to a large extent referable to the number of circulating leukocytes. If, therefore, with a given local inflammation the leukocyte level could at will be raised for a protracted period, a definite tool would be available to one in reenforcing antibiotics when dealing with a number of infectious processes.

In this communication data are collected to indicate that, with an intravascular injection of the leukocytosis-promoting factor (abbreviated as the LPF) and a concomitant pleural inflammation, the high level of leukocytes is maintained for longer intervals. The superimposition of this substance tends to reenforce the very mechanism which aids in the ultimate disposal of an irritant, namely, the rise in the number of circulating leukocytes.

EXPERIMENTS

Dogs were used and 1.5 cc. of turpentine was injected intrapleurally as described previously.⁴ By the next day acute pleurisy had developed, and the white blood cell level was elevated. At that time various amounts of canine leukocytosis-promoting factor ranging from 23 to 57 mg. dissolved in several cubic centimeters of isotonic solution of sodium chloride were introduced into the

This paper is no. 38 of a series entitled "Studies on Inflammation."

From the Agnes Barr Chase Foundation for Cancer Research, Temple University School of Medicine.

1. Menkin, V.: *Am. J. Path.* **16**:13, 1940; *Arch. Path.* **30**:363, 1940.
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3. Menkin, V.: *Arch. Path.* **41**:376, 1946.
4. Menkin, V.: *Am. J. Path.* **10**:193, 1934.

circulation. Hourly counts were taken for about six hours to determine the level of the circulating white cells. A white blood cell count was taken once daily for each subsequent day until the level of circulating white cells was again approximately similar to the basal level prior to the beginning of the experiment. The results of the observations are assembled in table 1. It is clear that the intra-

TABLE 1.—*The Effect of the Leukocytosis-Promoting Factor on the Level of Circulating Leukocytes with a Concomitant Local Inflammation*

Dog	Amount of LPF Injected into Blood at Time of Pleural Inflammation, Mg.	Basal White Blood Cell Count	White Blood Cell Level One Day After Development of Pleural Inflammation Alone	Maximal White Blood Cell Count Within Five Hours After Intravascular Injection of LPF at Time of Pleural Inflammation	Days Following Additional Intravascular Injection of LPF With Local Inflammation Before White Blood Cell Level Became Again Normal
38-T.....	23.0	8,650	27,950	39,300	23
30-T*.....	37.7	4,750	11,275	16,000	12
39-T.....	33.0	18,025	52,400	62,850	6
33-T.....	34.0	9,450	19,700	34,500	5
16-T.....	57.0	13,075	36,100	54,750	4
37-T.....	51.0	16,150	28,550	50,650	3
Average.....	39.3	11,683	29,329	43,008	9

* This animal had been given an intrapleural injection of the irritant about two months previously. This is the second injection of the irritant.

vascular superimposition of a dose of leukocytosis-promoting factor when there is a concomitant local acute inflammation increases markedly the level of circulating leukocytes. In the series of dogs studied the average basal white blood cell level amounted to 11,683. With an acute pleural inflammation, leukocytosis

TABLE 2.—*The Effect of an Acute Local Inflammation of the Pleura on the Leukocyte Level in the Circulation*

Dog	Basal White Blood Cell Count	White Blood Cell Level One Day After Intrapleural Injection of Irritant	Days Following Acute Inflammation of the Pleural Cavity Before White Blood Cell Level Became Again Normal
49-T.....	15,900	19,100	1
29-T.....	12,200	20,550	1
42-T.....	19,600	26,200	1
46-T.....	12,900	26,800	1
48-T.....	15,800	25,550	1
Average.....	15,280	23,640	1

ensued, so that the average count was 29,329. With the intravascular injection of the leukocytosis-promoting factors, the leukocytosis became more pronounced; the average white blood cell count was 43,008. This additional activation of the marrow by the injected leukocytosis-promoting factor caused the white blood cell level to remain elevated for periods ranging from three to twenty-three days, with an average of nine days. On the other hand, when controls were used which

received only the irritant (1.5 cc. of turpentine) in the pleural cavity, the leukocytosis failed to be sustained. About one day after the development of the acute pleural inflammation, the level of circulating leukocytes became again normal (table 2). This response is wholly different from the protracted effect seen when the administration of leukocytosis-promoting factor is added to an already existing inflammation (table 1). When the leukocytosis-promoting factor alone is given

TABLE 3.—*The Effect of a Single Intravascular Injection of Leukocytosis-Promoting Factor Alone on the Leukocyte Level in the Circulation*

Dog	Amount of LPF Injected into Blood, Mg.	Basal White Blood Cell Count	Maximal White Blood Cell Count Within 3 to 6 Hours After LPF Was Injected into Circulation	Days Following Intravascular Injection of LPF Before White Blood Cell Level Became Again Normal
9-T*	36	9,350	17,100	1
11-T*	51	11,000	21,200	1
8-D*	57	9,700	14,500	1
44-T	53	7,700	12,350	1
38-T	25	10,050	27,450	1
Average	44.4	9,560	18,500	1

* This animal had been given numerous injections of LPF in the past.



Graphic representation of the effects on the blood leukocyte count of: intra-vascular injection of leukocyte-promoting factor plus inflammation of pleura caused by intrapleural injection of turpentine (black columns); pleural inflam-mation alone (shaded columns); injection of leukocytosis-promoting factor alone (white columns).

to dogs, the level of circulating leukocytes remains elevated also for about one day (table 3). Typical experiments are illustrated in the accompanying figure. It is clear that when there is an inflammation in an animal and when in addition the leukocytosis-promoting factor is injected into the circulation, the number of circulating leukocytes is augmented for a period of about a week (figure), in

contrast to the short periods of elevation when there is either an inflammation alone or when the leukocytosis-promoting factor is injected only by itself. In such cases the number of circulating leukocytes remains high for approximately one day after the development of marked acute inflammation (figure). Observations were also made on 2 dogs which had previously received the leukocytosis-promoting factor and which several days later were given an intrapleural injection of turpentine. In these animals it seemed as if the activation of the bone marrow by the preliminary injection of the leukocytosis-promoting factor induced also a sustained response in the leukocyte level with the subsequent administration of the irritant. The counts in these 2 animals became normal only after four days. Thus, it would seem as if the leukocytosis-promoting factor administered either prior to the inflammation or after it is already in progress causes a rise in the leukocyte level which is maintained for several days longer than when there is just inflammation.

COMMENT

The foregoing observations clearly indicate that the number of circulating leukocytes can be increased and sustained at a high level when to an already existing inflammation an intravascular injection of leukocytosis-promoting factor is added. The combination of inflammation and injection of leukocytosis-promoting factor reenforces the natural leukocytosis which tends to develop with some types of inflammatory reaction. Since the prognosis of a number of inflammatory processes depends to some extent on the level of leukocytes⁵ and since the leukocytosis-promoting factor can be injected innocuously into human beings,³ herewith lies a factor which can be utilized in numerous clinical conditions in order to reenforce the antibiotics commonly used against various infectious processes.

SUMMARY AND CONCLUSIONS

The leukocytosis-promoting factor when injected into the blood stream of an animal with an already existing inflammation raises and maintains for a prolonged interval the number of circulating leukocytes. Under such circumstances the leukocytosis is sustained for several days longer than when there is an inflammation alone or when the leukocytosis-promoting factor is introduced without a concomitant inflammation. Some observations suggest also that this factor when injected several days prior to an acute inflammation of the pleura likewise tends to maintain a high leukocyte level in the blood for longer intervals. The clinical implications of these findings are discussed.

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EPIDERMoids (CHOLESTEATOMAS) OF THE BRAIN

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NEITHER the name "pearly tumor" (*tumeur perlée*), given to these neoplasms by Cruveilhier¹ because of their highly refractile and nodular external surface, nor "cholesteatoma," as Müller² called them for their cholesterol content, is satisfactory. Histogenesis offers a firmer basis for correct nosology. The cholesteatoma occurring in the middle ear (air cells of the mastoid process) is, according to most, a legacy of chronic inflammation and, histologically, is a mass of laminated eosinophilic material devoid of cellular elements. But Cushing³ believed even this to be a true epidermoid tumor which predisposed to secondary otitis media. Rand and Reeves⁴ recently suggested the possibility that tumors of this type may be examples of the larger class of diploic or cranial epidermoids. Cholesteatomas occurring in situations other than the middle ear are, by a general consensus, considered genuine neoplasms. Histogenetically, they are either epidermoids (the commoner type) or dermoids arising from ectopic fetal epidermal cell rests (Bostroem⁵; Critchley and Ferguson⁶; Munro and Wegner⁷; Rand and Reeves⁴), a view originally put forth by von Remak.⁸ "Cholesteatoma" is therefore an unfortunate designation, and these neoplasms should be termed epidermoids or dermoids depending on whether histologically they show pure ectodermal structures or whether, in addition to the epidermal lining, derivatives of the mesodermal corium can also be identified. The unequivocal demonstration of persistent squamous epithelium is, according to Love and Kernohan,⁹ a

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point of distinction between a true neoplasm and an inflammatory cholesteatomatous mass. Characteristically the epidermoids are composed of four concentric layers of varying thickness—the acellular stratum durum responsible for the pearly shine, the cellular stratum granulosum made up of cuboidal to stratified epithelium containing keratohyaline granules within the cytoplasm of its cells, the stratum fibrosum and the stratum cellulosum. The breaking down of keratin and keratohyalin produces the cholesterol crystals. The dermoids, on the other hand, show an outer fibrous layer—a connective tissue matrix which may contain sebaceous glands, hair follicles, fat cells, smooth muscle fibers, elastic tissue fibers and blood vessels—and an inner epithelial layer.

These neoplasms—the epidermoids and the dermoids—have been shown to originate beneath the scalp, within the diploe of the skull, in the meninges, in the substance of the brain and the spinal cord, and even from the choroid plexuses of the ventricles. The commonest site is the subarachnoidal cistern, near the midline, at the base of the brain, or in the region of the fourth ventricle. For the epidermoid a common site is the cerebellopontile angle (Rand and Reeves⁴). The epidermoids are usually of a solid consistency, but the dermoids are more apt to be cystic. Both of them usually occur singly, grow slowly (the average duration of symptoms being many years) and run a benign course. Recurrence is mostly due to incomplete resection of the wall, but at least 2 cases in which there was a secondary malignant change have been recorded; in Ernst's¹⁰ case a metastasizing carcinoma was the end result, and in Stromeyer's¹¹ case a sarcoma arose from the mesodermal element of the capsule. The symptoms and signs produced are varied and nonspecific; situated within the diploe the growth may produce a deformity and often a diagnostic roentgenogram, but if it occurs within the cranium, the symptom complex is that of increased intracranial tension, and the localizing signs are due to the effects of its pressure on the neighboring structures. There is no record of a single case of intradural epidermoid diagnosed prior to operation or autopsy (Love and Kernohan⁹). Not a few of these neoplasms have been accidental discoveries at the time of a carefully performed autopsy.

Verattus¹² described the first case of "cholesteatoma" (dermoid) of the brain, and up to 1936 some 56 cases of intracranial dermoid had been described (Courville and Kimball¹³). The epidermoid occurs more frequently. Rand and Reeves⁴ computed that fewer than 200

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cases of epidermoid of the central nervous system had been reported to date. The *Quarterly Cumulative Index Medicus* has not recorded any more cases of "cholesteatoma" of the brain to the end of 1945. It appears, therefore, that the so-called cholesteatoma of the brain is an uncommon neoplasm, accounting for less than 1 per cent of all kinds of intracranial growths.

REPORT OF CASES

CASE 1.—A Hindoo man aged 35 was admitted for (1) inability to use the left arm and the left leg—duration two years—and (2) headache—duration two months. The complaint started about two years prior to admission, with a feeling of weakness of the left foot. Gradually the weakness crept up to involve the whole of the limb. For the last several months he had used a stick to support himself, and for the last two months the limb had been practically useless. About six months after the onset of the first symptom he noticed weakness of the left hand, and this slowly spread up to involve the whole extremity. There was no subjective sensory disturbance of any kind in the affected limbs at any time. For the last two months he had suffered from headache in the frontal region. This was bilateral from its inception and in the beginning was irregular, with intervals of freedom, but for the last three weeks it had grown in intensity and had become constant. There was no history of vomiting, nor had he noticed any diminution of vision. As far as he remembered he suffered from no serious illness in his childhood. About six years ago he had a sore on the penis, which subsided without any specific treatment. He was a sweet-meat seller, subsisted on a vegetarian diet and smoked moderately; he had never tasted alcohol. There was nothing to note in the history of his family; he had five children, all healthy.

The general examination showed him to be a fairly well built and well nourished person. His temperature was 97 F.; pulse rate, 80; respiratory rate, 24. The conjunctivas and the nails were normal; the tongue appeared raw at the edges. The cervical lymph nodes of the right side were palpable, as also was the right epitrochlear gland; the inguinal nodes of both sides were palpable. Examination of the alimentary, respiratory and cardiovascular systems showed nothing abnormal. The blood pressure was 110 systolic and 70 diastolic. Psychologically he appeared to be a man of average intelligence and good memory. The neurologic observations were as follows: The pupils were central and equal but dilated; the reaction to light as well as to accommodation was sluggish. There was complete paralysis of the left side of the face; the other cranial nerves were normal. On the left side the motor power was diminished in both the upper and the lower extremities, more so in the latter. It was normal on the right side. The tone was increased on the left side, more so in the lower extremity. The right side appeared normal. The coordination, as far as could be judged, was unaffected. The left arm alone showed some wasting of the muscles; no test was made for the reaction of degeneration. The deep reflexes could be elicited on both the sides; all of those of the left side were markedly brisk. There was no patellar or ankle clonus. Among the superficial reflexes, the abdominal ones were present except in the left lower quadrant. The cremasteric reflex was absent on the left side. The plantar was flexor in type on the right side; on the left side it was extensor. The visceral reflexes were unaffected. The sensorium was normal. The gait was hemiplegic. Romberg's sign was observed.

Funduscopy showed bilateral papilledema. The field of vision of each eye was normal. Roentgenologic examination of the skull produced nothing distinctive.

Encephalography revealed defective visualization of the anterior horn of the right lateral ventricle. Ventriculography confirmed this finding: The right lateral ventricle was irregular, smaller and indistinct in outline; the left lateral ventricle was well seen but appeared displaced to the left.

Laboratory Investigations.—The blood revealed moderate normocytic, normochromic anemia. The leukocytic count was within the normal range. Both the Kahn and the Wassermann test were negative. The blood group was O.

The cerebrospinal fluid was under tension; manometry was not done. The fluid was clear. The total protein content was 200 mg. per hundred cubic centimeters; a test for globulins was positive; the sugar content was 60 mg. and the chloride content 680 mg. per hundred cubic centimeters; the cell count showed 3 cells per cubic millimeter, all lymphocytes; the Wassermann reaction was negative.

Gastric analysis showed a hypochlorhydric type of curve. The urine and the feces showed no abnormality.

Further Progress in the Wards.—The clinical diagnosis was "intracranial growth of the cerebrum, right side." An operation for decompression was undertaken. At operation the membranes were seen to be tense, and on incision the brain tissue protruded. The gyri were flattened, and the brain substance underneath felt firm. On incising the covering brain matter, one could see the neoplasm in the depths: it appeared as glistening white tissue, practically avascular and friable. The lesion appeared to be too extensive for any radical procedure, and only a small piece was removed for biopsy. The patient was in a state of severe shock after the operation. For this he was treated by injection of dextrose-saline solution and plasma and a blood transfusion. He was in a semiconscious state for two days and became deeply unconscious on the third day. His temperature shot up to 104 F., and he died on the fourth day after the operation. Permission to perform a full autopsy was refused, but permission to remove the brain was granted.

The Specimen (fig. 1).—The brain weighed 1,610 Gm. The membranes were normal except in the field of the surgical procedure. On separating them one saw that the gyri were flattened. The vessels on the surface of the brain were hyperemic. The organ was asymmetric and deformed; the right hemisphere of the brain was more bulky than the left one. The lesion appeared to be confined to the cerebral hemispheres; the cerebellum, the pons and the medulla were normal. The cut surface showed the following features: The neoplasm formed a large irregular mass measuring 8.5 by 8 by 6.5 cm. in the longest diameters. The greater part of it was situated centrally and extended into the cerebral hemispheres at the sides—to within 3 cm. of the left lateral surface of the cerebrum and to within 1.5 cm. of the right. Serial sections showed that superiorly on the right side it had nearly come to the surface, but that on the left side a width of 3 cm. of brain substance still lay intact. Inferiorly in the parieto-occipital region it had reached the deepest—to within 2 cm. of the base of the brain. Viewed as a whole, the bulk of it lay in the parieto-occipital region, but a small offshoot, measuring 2 by 1.5 cm., projected into the right frontal lobe. A membranous capsule could be traced all round, and with the naked eye one could see no definite infiltration of the brain substance at its margins. The impression produced was that the neoplasm had hollowed out for itself a bed in the substance of the brain tissue. In this process it had encroached on the major portion of the right lateral ventricle, leaving just a small part of the anterior horn still intact. On the left side, the body of the lateral ventricle alone was occupied, and the anterior and inferior

horns were free. All the structures in the wide compass of its extent were, naturally, destroyed. The neoplasm was apparently soft and friable, because many bits from it were easily dislodged and fell off during the sectioning. The cut surface was pale grayish white and grayish yellow; at many places were specks and streaks and even small irregular islets of tissue, which were glistening and pearly looking. The matter composing the neoplasm formed large irregular flakes, held loosely together. The brain substance all round the neoplasm was evidently compressed and both the cerebral hemispheres irregularly deformed. It may be stated, in retrospect, that the biopsy material sent for examination had sufficient distinctive features like the parent neoplasm to suggest its cholesteatomatous nature, and the scraping made from it and examined as a cover slip preparation showed cholesterol crystals.

Histologic Examination (fig. 2).—Most of the tissue consisted of cholesteatomatous matter in which coarse strands showed eosinophilic staining and were disposed in a wavy manner. No cellular elements could be detected in this. Clefts of cholesterol crystals could be seen all over. A large number of blocks had to be cut from different portions of the neoplasm before the epidermal lining could be demonstrated. The latter showed all the different layers—the stratum durum, the stratum granulosum, the stratum fibrosum and the stratum cellulosum. None of the sections showed any derivatives of the corium.

CASE 2.—A Hindoo man aged 25 years complained of pain in the left half of the face—duration six months. The onset was insidious, with pain localized to the angle of the mouth on the same side in the beginning. The pain was, according to him, deep seated, growing, irregular in occurrence and exacerbated by any movement of the part. It had gradually increased in intensity and spread over a wider area to involve, in turn the inside of the mouth, the lower jaw, the face and lastly the scalp. All along, it had been strictly confined to the left half of the face. For the last two weeks it had become excruciating and of a lightning-like character; it was now made worse by the slightest movement of the face and was persistent, with no intervals of freedom. There was no history of headache or vomiting. There was nothing significant in his past history or in the family's history. He was a man of moderate habits and earned his bread at a clerical job.

At this stage—after suffering for about six months—he consulted a surgeon outside the hospital. The relevant details extracted from the surgeon's notes are as follows: The patient was in extreme agony at the time of examination. The only physical sign to note on local examination was the tenderness all over the left half of the face. Examination of the nervous system gave negative results. The other body systems were normal. Funduscopy was not done. The surgeon's diagnosis was "trigeminal neuralgia." Six days before the patient sought admission to the hospital the surgeon attempted to inject alcohol into the trigeminal ganglion. Within a few minutes after the injection there developed marked swelling of the lids of both eyes, redness of the left half of the face and bilateral ptosis. Both pupils were dilated, and there was no reaction to light or accommodation. The pain continued unabated as before. He was immediately transferred to the King Edward VII Memorial Hospital, Bombay.

Examination on admission showed a well built and fairly well nourished man. His temperature was 99 F.; pulse rate, 110; respiratory rate, 26. The look was anxious, and the man appeared to be overwhelmed by the pain. The eyelids of both eyes were moderately swollen, and the skin over the left half of the face was reddened. The left eye alone showed ptosis, and its upper eyelid was redder than the rest of the face on that side. The left pupil was semidilated and reacted

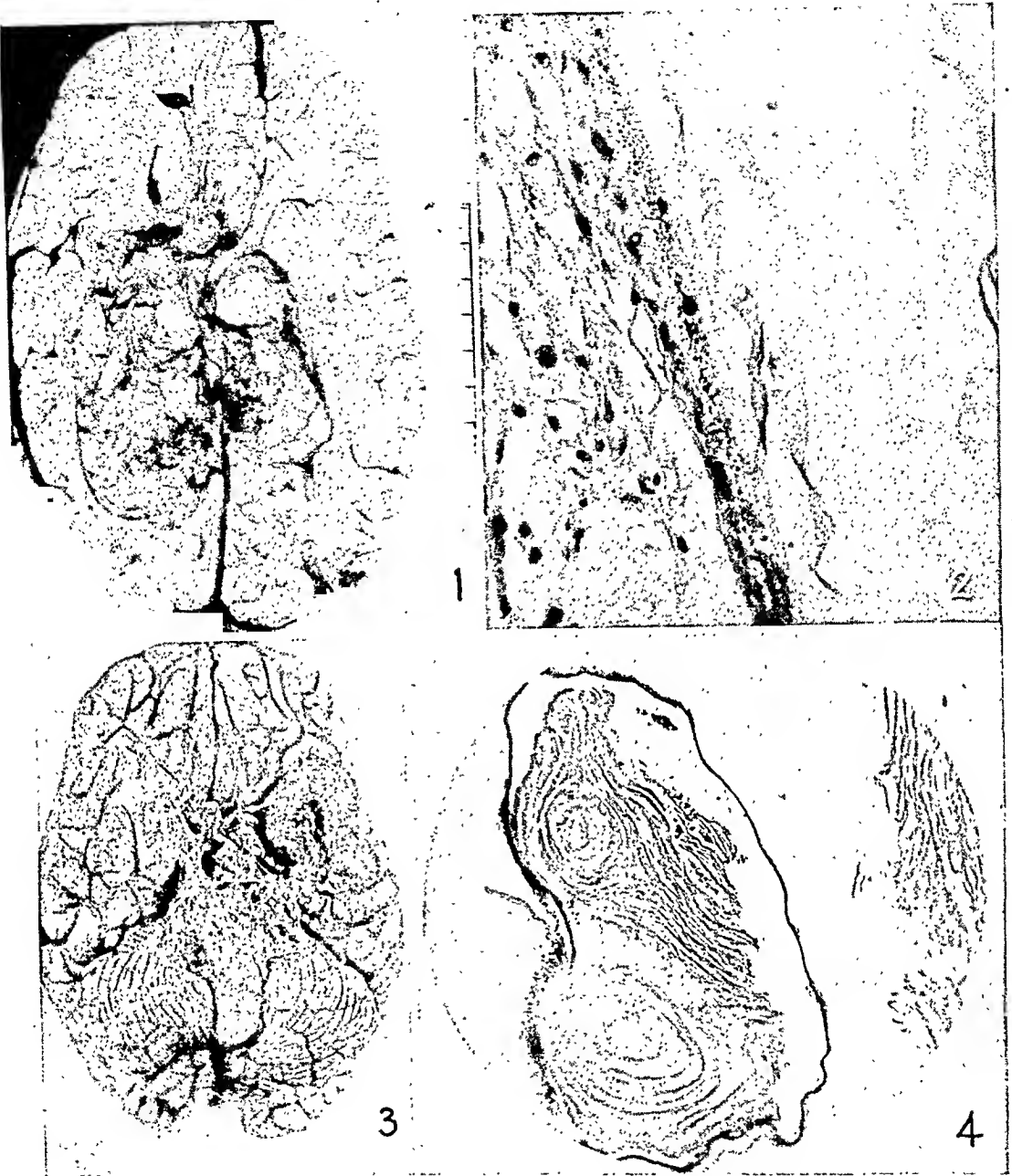


Fig. 1 (case 1).—Specimen of the brain—the cut surface of the lower half. It shows the extent and the general features of the neoplasm.

Fig. 2 (case 1).—Photomicrograph of a histologic section of the specimen. The stratum granulosum with the keratohyaline granules in its cells can be easily seen. $\times 200$.

Fig. 3 (case 2).—Specimen of the brain showing the main mass of the neoplasm lying in the recess between the pons and the cerebellum, on one side, and the temporal lobe, on the other. The latter shows raw areas (operative injury).

Fig. 4 (case 2).—Photomicrograph of a histologic section of the specimen. Note the epidermal lining all round an islet of cholesteatomatous tissue. Hematoxylin and eosin; $\times 70$.

sluggishly to light; the reaction to accommodation was normal. The right pupil showed no abnormality. The left half of the face was tender all over. The sensations of touch, pain and pressure were clearly elicited over the right half of the face, but over the left half they seemed somewhat dulled. The rest of the nervous system was normal and the other body systems showed no changes. The dental surgeon reported the gums to be pyorrheic but could find no carious or tender teeth. An overhaul by the ear, nose and throat department revealed no abnormality. Unfortunately, even at this stage, funduscopy was not done.

Laboratory Investigations.—Cytologic examination of the blood gave a normal count. The Kahn test was positive. The urine and the feces showed no abnormalities. The cerebrospinal fluid was under tension. The chemical and cytologic examinations showed no deviations from the normal.

Further Progress in the Wards.—The clinical diagnosis was "trigeminal neuralgia." The pain continued, and it was decided to perform Frazier's operation of trigeminal ganglionectomy. The ganglion was successfully resected accordingly; nothing eventful was noticed at the operation. However, after the operation the patient never regained consciousness. He gradually became comatose, a high temperature of 105 F. developed and death occurred within twelve hours after the operation. The permission for autopsy allowed only removal of the brain.

The Specimen (fig. 3).—The brain weighed 1,250 Gm. The membranes were hyperemic all over. There was no flattening of the gyri or deepening of the sulci. The brain substance proper appeared redder than normal. There was no deformity of the cerebrum as seen from its superior and lateral surfaces. Examination of the base of the brain showed that the left temporal lobe was damaged. Here the surface was uneven, at places raw, and large blood clots were clinging to the brain tissue. After these had been gently removed, the brain substance beneath was found depressed and flattened. In addition, there could be seen now a grayish white and grayish yellow refractile tissue distributed irregularly over the inferior surface of the left temporal lobe and the left half of the pons along its inferior and lateral surfaces. The major portion of this mass was, however, lodged in the recess between the pons and the cerebellum, on one hand, and the adjoining portion of the left temporal lobe, on the other. The exact extent of this mass was difficult to decide; its original relationship appeared to have been disturbed by the operative procedures. It formed, however, an irregular, lobulated mass measuring 4 by 3 by 2 cm. in its widest diameters. It seemed to be situated intradurally in the subarachnoid space. It was friable, small bits being easily dislodged even by gentle handling. Both in the fresh and in the fixed specimen, the impress of the tumor on the adjoining surfaces of the pons and the temporal lobe of the left cerebral hemisphere was evident. The disposition and the extent of the neoplasm as judged even from its remnants indicated that it could have produced the interesting symptom complex by pressure either on the ganglion of the trigeminal nerve or on the sensory root of the trigeminal nerve.

Histologic Examinations (fig. 4).—Most of the material consisted of cholesteatomatous matter. After a prolonged search of the sections from different portions, one bit was found to show the epidermal lining. This formed a thinned layer of three to five rows of cells; the stratum durum and the stratum granulosum could be identified.

CASE 3.—A Hindoo man aged 21 years was admitted with the history of having met with a serious tramcar accident. He was in a state of advanced surgical shock. There was a compound fracture of the bones of the left leg and a crushing injury of the right foot. He died within three hours after admission.

Autopsy.—All the injuries mentioned in the clinical notes were confirmed. Beyond the changes in viscera, produced by shock, there was nothing to note. The brain showed at its base a small neoplasm. This was situated intradurally and was adherent by a tiny tag to the right cerebellar peduncle and in the same way to the petrous portion of the temporal bone. The whole organ was carefully dissected out and fixed.

The Specimen (fig. 5).—The brain weighed 1,200 Gm. The meninges were normal. The gyri and the sulci did not show changes. At the base, in the right cerebellopontile angle, was seen an irregular, freely mobile mass measuring 3 by 2.5 by 2 cm. It seemed to lie in the subarachnoid space. It was well circumscribed and covered by a thin capsule. The brain substance was quite free. The under surface of the cerebellum was slightly indented by the mass, and its impress on the lateral surface of the pons was even more shallow.

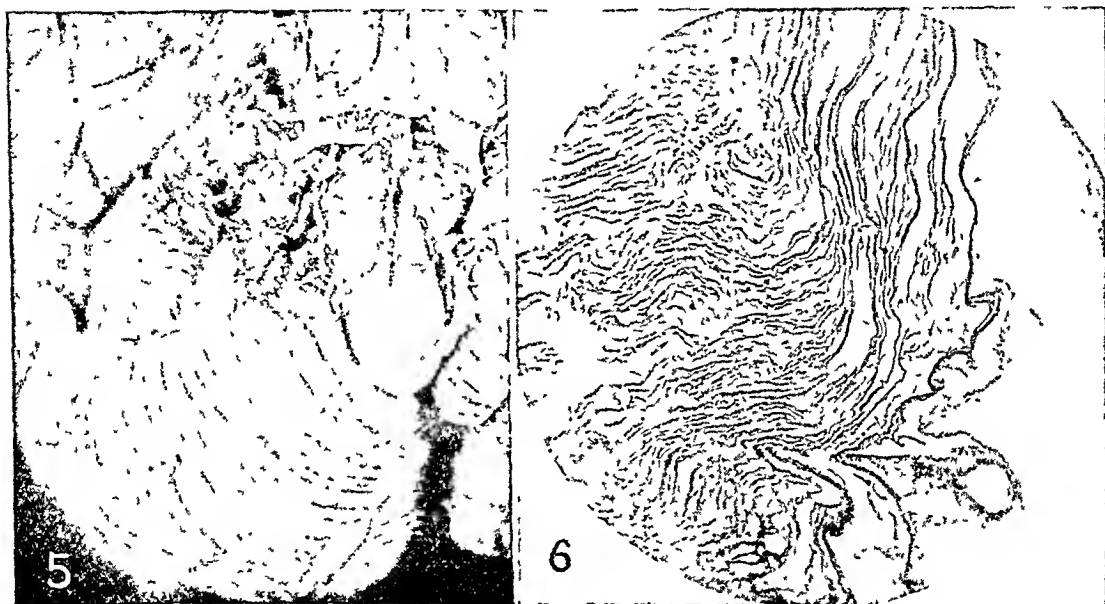


Fig. 5 (case 3).—Specimen of the brain showing the neoplasm lying in the right cerebellopontile angle.

Fig. 6 (case 3).—Photomicrograph showing the cholesteatomatous tissue with the epidermal lining on the surface. Hematoxylin and eosin; $\times 70$.

Histologic Examination (fig. 6).—Most of the tissue was fibrillar and cholesteatomatous in nature. Many of the bits, however, showed a thinned out epidermal lining. This consisted of two to four layers of polyhedral cells with dark oval nuclei and eosinophilic nongranular cytoplasm. The outer surface of this lining showed keratinization at places, but nowhere could elements of the corium be demonstrated.

COMMENT

The unequivocal demonstration of an epidermal lining in all the 3 tumors would differentiate them as true neoplasms (Love and Kernohan⁹). No derivatives of the corium were detected, and histologically, therefore, the neoplasms were epidermoids. That in the first case had

grown to such a large size that its exact origin could not be told; its situation, however, suggested its seat to be some midline structure. The production of the characteristic symptoms of an "intracranial growth" could be easily appreciated. In the second case the neoplasm had produced pronounced pressure symptoms to delude the clinician into a diagnosis of trigeminal neuralgia. There was neither headache nor vomiting. The omission of a fundusoscopic examination was most unfortunate. This investigation might have been helpful in reaching the correct diagnosis, and in the absence of any data from this source it would be incorrect to state that the general symptoms of an "intracranial growth" were not present. The neoplasm in this case was situated in the subarachnoid space and was meningeal in origin. In the third case the neoplasm arose from the meninges and was discovered at autopsy. The history subsequently elicited, if relied on, would suggest that the growth had produced no symptoms during life.

SUMMARY

Three cases of epidermoid of the brain are reported.

In the first case the symptoms and signs of an "intracranial growth" were evident enough to enable one to reach the correct diagnosis. In the second case the neoplasm produced peculiar pressure symptoms, and the case was misdiagnosed as one of trigeminal neuralgia. In the third case the neoplasm was an incidental finding at autopsy.

NODULAR INFLAMMATORY AND DEGENERATIVE LESIONS OF MUSCLES FROM FOUR HUNDRED AND FIFTY AUTOPSIES

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AND

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MINNEAPOLIS

IT HAS been an interesting observation that rheumatic and rheumatoid inflammations tend to be located in nodules in various tissues of the body. The terms "Aschoff nodules," "subcutaneous nodules," "Masson bodies" and "nodular polymyositis" are commonly noted in the medical literature.

Rheumatic nodular inflammation has been observed and described in the subcutaneous tissues,¹ the joints, the tendons, the diaphragm, the tongue and other muscles,² the galea aponeurotica,³ the tonsils,⁴ the arteries,⁵ the valves,⁶ atriums⁷ and ventricles⁸ of the heart and the lungs.⁹ Subcutaneous nodules and similar inflammatory processes have repeatedly been seen and described in association with the state of rheumatoid arthritis.¹⁰

In 1928 Maclachlan and Wayne⁴ studied the tonsils and the muscles of the tongue and tonsillar region in cases of acute rheumatic

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fever. Between the muscles he found an infiltrate of lymphocytes, plasma cells and endothelial cells. When muscle fibers had become atrophic, they were invaded by endothelial leukocytes, and multinucleated giant cells were noted.

Curtis and Pollard¹¹ studied biopsy specimens of calf muscles in cases in which chronic arthritis was included in Felty's syndrome. They observed areas of interstitial and perivascular infiltration of lymphocytes.

Our interest in the character and the frequency of muscular inflammatory nodular areas and the degrees of muscular degeneration in cases of rheumatoid arthritis and acute rheumatic fever was stimulated by the recent thorough study of these muscular lesions by Steiner and his co-workers.¹² They studied the muscles in 9 cases of rheumatoid arthritis. The muscles had been obtained for biopsies. In the nodular inflammatory areas Steiner found lymphocytes and plasma cells to be abundant. Polymorphonuclear leukocytes and eosinophils were rare or absent. These nodules were located in the endomysium and the perimysium. In the larger nodules an epithelioid type of cells was seen. The nodules were found in each of the 9 cases.

In a study of 44 cases of rheumatoid arthritis with Wells and Wetherby,¹³ biopsy specimens were obtained from deltoid muscles, and lesions were found similar to those described by Steiner, in 17 (38.6 per cent) of the cases.

Steiner also emphasized the degenerative processes which occurred in association with the nodular inflammatory areas. The degenerative changes noted in the muscles consisted of atrophy and various degrees of necrosis. Fatty metamorphosis and hydropic degeneration were noted. The muscular nuclei were increased in number, size and shape and stained more deeply than normal. It was Steiner's opinion that the degenerative changes were secondary to the inflammation. In 11 of our 44 cases one or more of degenerative processes were noted in the deltoid muscle.

In a control study of 196 specimens taken from routine autopsies the nodular myositis, the perineuritis and the degenerative nuclear changes were not noted by Steiner.

The purpose of the present paper is to furnish controls for our previous observations on biopsy specimens of muscles in cases of rheumatoid arthritis and to obtain further information concerning inflammation and degeneration in a greater number of cases and in a larger number of muscles than could be obtained by biopsies.

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Seven muscles were collected from each of 450 autopsies and studied for evidences of inflammation and various stages of degeneration. The muscles studied were the pectoral, the sternocleidomastoid, the deltoid, the diaphragm, the intercostal, the psoas and the sacrospinalis.

OBSERVATIONS

Inflammatory Lesions.—The nodular inflammatory areas were graded in respect to size and frequency from 1 to 4 plus. One plus

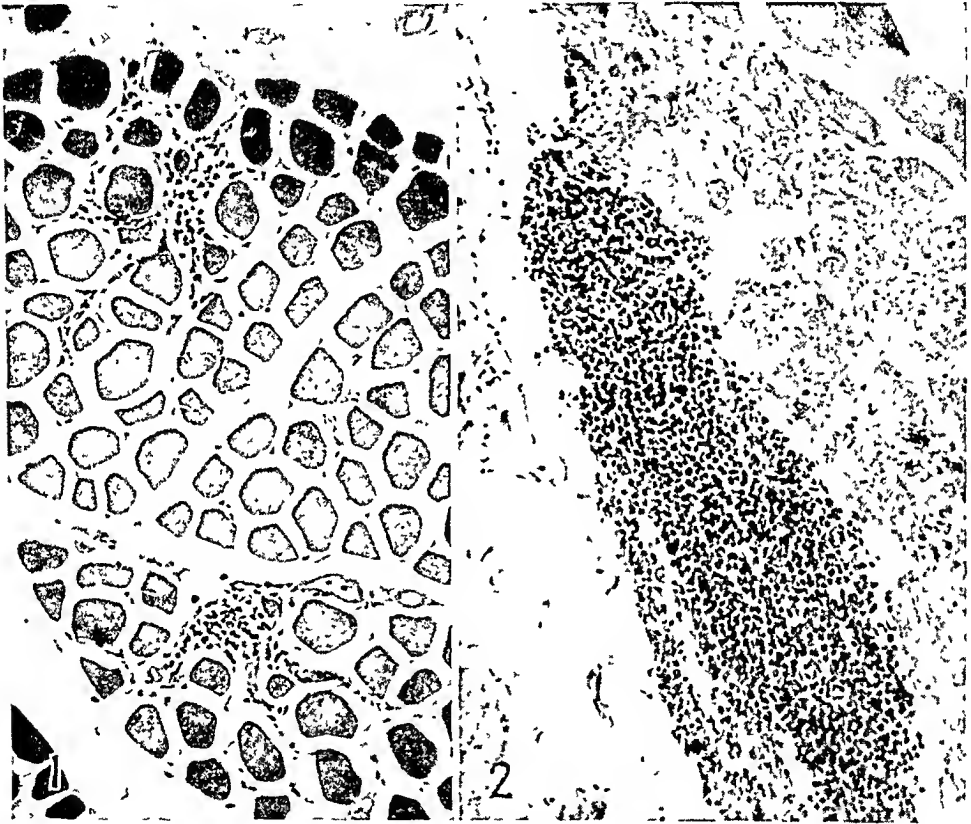


Fig. 1.—Inflammatory nodules (1 plus grade) between muscle fibers.

Fig. 2.—Inflammatory nodule (4 plus grade) in interstitial tissue.

represented a small nodule (fig. 1). The presence of but a few lymphocytes was not recorded. The presence of one or more nodules in a section was also considered in evaluating the grade. A 4 plus grade represented one or more large nodules or several smaller ones in the same section (fig. 2.). A 2 plus and a 3 plus grade were intermediate.

The inflammatory nodules consisted primarily of infiltrating lymphocytes and plasma cells. A few polymorphonuclear leukocytes were occasionally seen, and in a few cases they were the principal cells. In many of the nodules the cells were more or less oval in shape, and

definite macrophages were sometimes seen. Some of these resembled epithelioid cells. In a few cases carcinoma had metastasized to the muscles. In a case of lymphatic leukemia there was a diffuse infiltration of lymphocytes.

The frequency and the degree of the inflammatory processes in the various muscles in the 450 cases are recorded in table 1. In 118 (26.2

TABLE 1.—*Frequency and Degrees of Nodular Myositis*
(*Four Hundred and Fifty Cases*)

Muscle	Cases Examined	Cases in Which Myositis Was Graded as Shown									
		+		++		+++		++++		+ or More	
		No.	%	No.	%	No.	%	No.	%	No.	%
1. Pectoral.....	450	10	2.2	7	1.5	0	...	0	...	17	3.7
2. Sternocleidomastoid.....	450	10	2.2	4	0.8	3	0.6	3	0.6	20	4.4
3. Deltoid.....	450	6	1.3	12	2.6	1	0.2	0	...	19	4.2
4. Diaphragm.....	450	16	3.5	25	5.5	14	3.1	6	1.3	61	13.5
5. Intercostal.....	450	10	2.2	16	3.5	5	1.1	5	1.1	36	8.0
6. Psoas.....	432	8	1.8	3	0.6	2	0.4	3	0.6	16	3.7
7. Sacrospinalis.....	150	4	2.6	5	3.3	0	...	0	...	9	6.0

Cases in which myositis of 1 plus or more was observed in one or more muscles—118 (26.2%)

TABLE 2.—*Frequency and Degrees of Atrophy of Muscles*
(*Four Hundred and Fifty Cases*)

Muscle	Cases Examined	Cases in Which Atrophy Was Graded as Shown									
		+		++		+++		++++		+ or More	
		No.	%	No.	%	No.	%	No.	%	No.	%
1. Pectoral.....	450	14	3.1	3	0.6	0	...	1	0.2	18	4.0
2. Sternocleidomastoid.....	450	28	6.2	4	0.8	1	0.2	0	...	33	7.3
3. Deltoid.....	450	23	5.1	12	2.6	0	...	1	0.2	36	8.0
4. Diaphragm.....	450	90	20.0	34	7.5	1	0.2	0	...	125	27.7
5. Intercostal.....	450	33	7.3	4	0.8	1	0.2	0	...	38	8.4
6. Psoas.....	432	24	5.5	5	1.1	0	...	1	0.2	30	6.9
7. Sacrospinalis.....	150	16	10.6	3	2.0	2	1.3	0	...	21	14.0

Cases in which atrophy of 1 plus or more was observed in one or more muscles—191 (42.4%)

per cent) of the 450 cases inflammatory lesions were observed of one or more grades and in one or more muscles. The diaphragm and the sacrospinalis muscle were most commonly affected. As seen in table 5, there were 83 cases in which one muscle was involved; two muscles were involved in 24 cases, three muscles in 6 cases, four muscles in 2, five muscles in 1 and six muscles in 2. In no instance was there involvement of all seven muscles.

Degenerative Processes.—The degenerative processes consisted of atrophy, Zenker's degeneration, necrosis and an increase in the number and a change in the shape and the size of the muscular nuclei. The atrophic muscular fibers were markedly shrunken as compared with

normal fibers in the same section (fig. 3). The fibers showed various degrees of loss of striations. Some of the fibers were swollen with complete absence of both cross and longitudinal striations. Calcification of the fibers sometimes was noted. The sarcoplasm often was lysed and had completely disappeared. The nuclei were frequently greatly increased in number. They varied decidedly in size and shape and were hyperchromatic (fig. 4). These three degenerative changes were each graded 1, 2, 3 and 4 plus. The various degenerative processes

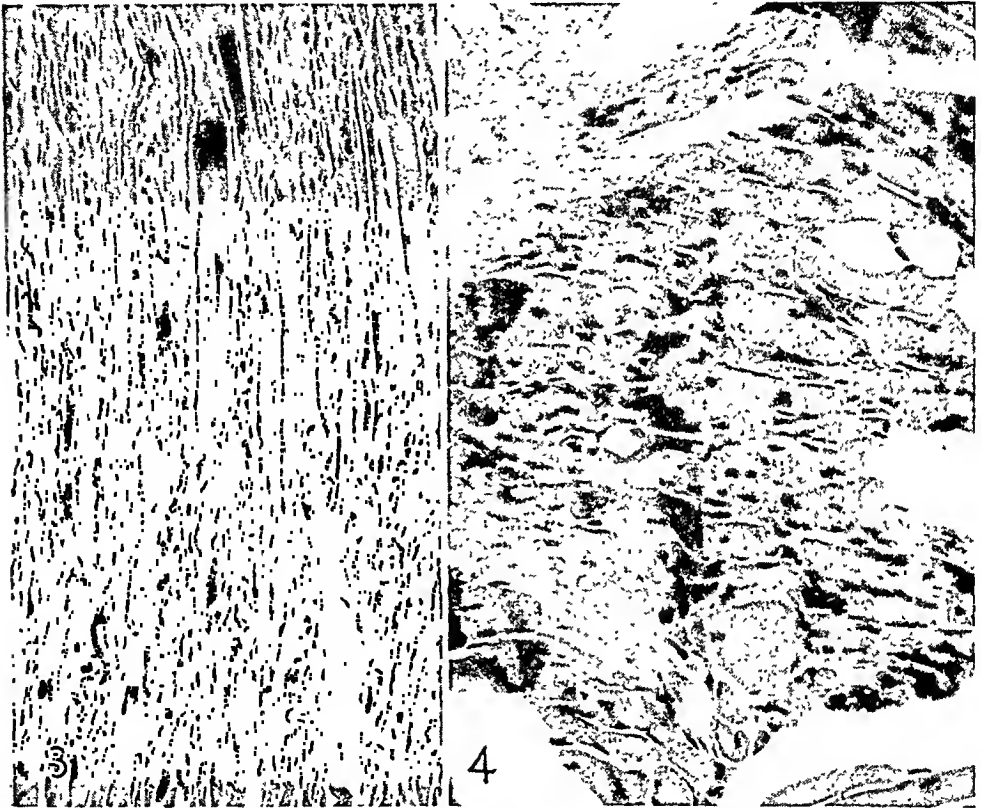


Fig. 3.—Atrophy of muscle with connective tissue replacement.

Fig. 4.—Nuclear enlargement and deformity.

observed in the muscles of the 450 cases reported in this paper are recorded in tables 2, 3 and 4.

Atrophy varying from slight narrowing to almost complete disappearance of the fibers was a common finding. The different degrees of atrophy and the frequency of involvement of the seven different muscles are recorded in table 2. Atrophy was present to some degree in all the muscles but was most pronounced in the diaphragm and the sacrospinalis muscle. In 191 of the 450 cases (42.4 per cent) there was atrophy of 1 plus or more in one or more muscles. The combination of muscular involvement per autopsy examined is recorded in table 5.

The frequency and the degrees of degenerative changes exhibited by cytoplasm varying from loss of striation to waxy degeneration and finally to complete necrosis of muscle fibers are tabulated in table 3. As with the inflammatory lesions, the greatest amount of change was noted in the diaphragm (17.5 per cent). The sternocleidomastoid muscle ranked next but was not much more commonly involved than the

TABLE 3.—*Frequency and Degrees of Sarcoplasmic Changes in Muscles*
(Four Hundred and Fifty Cases)

Muscle	Cases Examined	Cases in Which Sarcoplasmic Changes Were Graded as Shown									
		+		++		+++		++++		+ or More	
		No.	%	No.	%	No.	%	No.	%	No.	%
1. Pectoral.....	450	5	1.1	2	0.4	1	0.2	0	...	8	1.7
2. Sternocleidomastoid.....	450	25	5.5	8	1.7	1	0.2	0	...	34	7.5
3. Deltoid.....	450	21	4.6	6	1.3	1	0.2	1	0.2	29	6.4
4. Diaphragm.....	450	62	13.7	13	2.8	4	0.8	0	...	79	17.7
5. Intercostal.....	450	21	4.6	2	0.4	1	0.2	0	...	24	5.3
6. Psoas.....	432	14	3.2	3	0.6	0	...	1	0.2	18	4.1
7. Sacrospinalis.....	150	3	2.0	2	1.3	1	0.6	0	...	6	4.0

Cases in which sarcoplasmic changes of 1 plus or more were observed in one or more muscles—152 (33.7%)

TABLE 4.—*Frequency and Degrees of Muscular Nuclear Changes*
(Four Hundred and Fifty Cases)

Muscle	Cases Examined	Cases in Which Nuclear Changes Were Graded as Shown									
		+		++		+++		++++		+ or More	
		No.	%	No.	%	No.	%	No.	%	No.	%
1. Pectoral.....	450	39	8.6	8	1.7	1	0.2	1	0.2	49	10.8
2. Sternocleidomastoid.....	450	33	7.1	6	1.3	1	0.2	0	...	39	8.6
3. Deltoid.....	450	32	7.1	19	4.2	1	0.2	1	0.2	53	11.7
4. Diaphragm.....	450	48	10.6	9	2.0	2	0.4	0	...	59	13.1
5. Intercostal.....	450	42	9.3	7	1.5	4	0.8	0	...	53	11.7
6. Psoas.....	432	25	5.7	7	1.6	2	0.4	0	...	34	7.8
7. Sacrospinalis.....	150	11	7.3	4	2.6	2	1.3	0	...	17	11.3

Cases in which nuclear changes of 1 plus or more were observed in one or more muscles—158 (35.1%)

other muscles with the exception of the pectoral muscle, which showed much less evidence of degenerative processes. In 152 of the 450 cases (33.7 per cent) there was atrophy of 1 plus or more in one or more muscles examined (table 5).

The nuclear changes observed showed several variations. The nuclei were often greatly increased in number. They varied in size and shape. Some became much elongated; some became enlarged and took on irregular shapes. The nuclei stained darkly.

The frequency and the degree of the nuclear changes (also graded 1, 2, 3 and 4 plus) of the seven muscles per autopsy examined are seen in table 4. The greatest amount of change, as with inflammation, atrophy

and sarcoplasmic degeneration, was noted in the diaphragm (13.1 per cent); but the pectoral, deltoid, intercostal and sacrospinalis muscles were almost as commonly involved. Nuclear changes of 1 plus or higher grade were observed in one or more muscles in 158 of the 450 cases (table 5).

Correlation of the Clinical Histories and the Inflammatory Lesions.

—In 312 of the 450 cases studied histories were available at the time of

TABLE 5.—*Combinations of Muscular Involvement*

Muscles Involved in Each Case	Cases in Which Given Abnormality Was Observed							
	Myositis		Atrophy		Sarcoplasmic Changes		Nuclear Changes	
	No.	%	No.	%	No.	%	No.	%
1.....	83	70.33	126	65.96	116	76.31	79	50.00
2.....	24	20.33	39	20.41	26	17.10	41	25.94
3.....	6	5.03	13	6.80	5	3.28	17	10.75
4.....	2	1.69	6	3.14	4	2.63	13	8.22
5.....	1	0.84	3	1.57	0	5	3.16
6.....	2	1.69	2	1.04	0	2	1.26
7.....	0	2	1.04	1	0.65	1	0.63
Total.....	118	99.96	191	99.96	152	99.97	153	99.96

Cases in which one or more lesions were observed in one or more muscles—293 (65.1%)

TABLE 6.—*Myositis—Age and Sex Incidence (Three Hundred and Twelve Cases)*

Decades	Males			Females			Total		
	No.	No. with Myositis	% with Myositis	No.	No. with Myositis	% with Myositis	No.	No. with Myositis	% with Myositis
1	13	4	30.7	9	2	22.2	22	6	27.2
2	5	0	10	3	30.0	15	3	20.0
3	8	0	12	3	25.0	20	3	15.0
4	11	5	45.4	9	2	22.2	20	7	35.0
5	7	2	28.5	10	3	30.0	17	5	29.4
6	34	12	35.2	20	5	25.0	54	17	31.4
7	43	12	27.9	13	5	38.4	56	25	44.6
8	42	13	30.9	22	8	36.3	64	21	32.8
9	19	0	21	5	23.8	40	5	12.5
10	3	0	1	0	4	0
Total	185	48	25.9	127	36	28.3	312	92	29.4

the analysis. The age and the sex distribution and the types of diseases were studied in an attempt to determine whether any relation existed between any of these factors and the frequency and the degree of the nodular inflammatory lesions of the seven muscles of each case studied.

The age and sex incidence is recorded in table 6. The greatest number of males fall into the sixth, seventh and eighth decades and the greatest number of females into the sixth, seventh, eighth and ninth decades. The highest percentage of positive findings of inflammatory lesions is in the seventh decade in the entire group, but the percentage of positive findings is almost as great in the sixth and eighth decades.

The outstanding facts to be noted from this analysis of age and sex incidence are that the incidence of nodular myositis is about equal in the two sexes and that it is greater in both sexes in the later decades.

The types of disease mainly responsible for the deaths in the 312 cases analyzed and the relative frequency of myositis in each of the groups of diseases are listed in table 7.

TABLE 7.—*Myositis in Different Types of Diseases*
(Three Hundred and Fourteen Cases)

Disease	Cases	No. with Myositis	% with Myositis
Heart diseases			
Acute rheumatic fever.....	6	6	100.0
Valve deformities.....	21	4	19.0
Bacterial endocarditis.....	5	2	40.0
Syphilis.....	1	0
Hypertension.....	24	7	29.1
Coronary sclerosis.....	31	8	25.8
Cor pulmonale.....	5	0
Total.....	93	27	29.0
Noninfectious diseases			
Accidents and trauma.....	43	11	25.5
Tumors.....	46	18	39.1
Cerebral hemorrhage.....	15	3	20.0
Cirrhosis of liver.....	9	5	55.5
Poisoning.....	5	0
Gastrointestinal conditions.....	11	5	45.4
Stillbirth or neonatal conditions.....	4	1	25.0
Rupture of aneurysm.....	3	1	33.3
Thrombosis or embolism.....	1	1	100.0
Diabetes.....	6	1	16.6
Anemia.....	1	0
Total.....	144	46	31.9
Infectious diseases			
Poliomyelitis.....	22	2	9.0
Tuberculosis.....	16	4	25.0
Infections of bladder and kidneys.....	14	2	14.2
Pneumonia.....	7	1	14.2
Abscesses.....	6	0
Peritonitis.....	4	1	25.0
Meningitis and encephalitis.....	2	0
Influenza.....	1	0
Lupus erythematosus.....	1	1	100.0
Bacteremia.....	1	0
Cholecystitis.....	3	1	33.3
Total.....	77	12	15.5

In table 7 are seen, first, the different diseases of the heart, with the percentage of cases of each in which there were inflammatory lesions of the heart muscle. There were 6 cases of acute rheumatic endocarditis in all of which inflammatory lesions were observed in one or more of the seven muscles. In only 4 of the 21 cases of valvular deformities resulting from previous rheumatic infections was there any evidence of myositis. In 2 of the 5 cases of subacute bacterial endocarditis there was myositis. There was only 1 case in which cardiac failure was due to syphilitic aortitis. No muscular lesions were observed in this case. In the hypertensive cases and cases in which death was

due to coronary sclerosis or thrombosis the frequency of muscular lesions was high, 29.1 per cent and 25.8 per cent, respectively. No muscular inflammatory lesions were present in the 5 cases of cor pulmonale. In 27 (29.0 per cent) of the 93 cardiac cases the nodular muscular lesions were present in some degree in one or more of the seven muscles examined. Two observations worthy of note in these cardiac cases are that muscular lesions are high in patients with acute rheumatic endocarditis, the young patients, and in patients with hypertension and coronary sclerosis, the older patients.

The second group of diseases shown in table 7 comprises noninfectious conditions. There are 144 cases. In 46 (31.9 per cent) the muscular lesions were observed. The incidence was high in patients dying of accidents and trauma, tumors, cirrhosis of the liver and gastrointestinal diseases. Nothing of relative importance is suggested in this group except that many of the positive findings are in the older people. The same was observed in the cardiac group.

A group of 77 cases in which infection of one kind or another, not including infectious heart diseases, was the chief cause of death is recorded last in table 7. In only 12 (15.5 per cent) of these was myositis present. The incidence was fairly high in cases of tuberculosis and in cases of renal infection (pyelonephritis, glomerulonephritis, etc.). An interesting observation is that muscular lesions were infrequent in the cases in which death was due to acute poliomyelitis; the patients were mostly young people. In the single case of lupus erythematosus six of the seven muscles were extensively involved. This group in general included younger people than the cardiac and noninfectious groups.

Association of Inflammatory and Degenerative Lesions.—Steiner, in his studies, found what he believed to be a definite relationship between nodular polymyositis and degeneration and atrophy of muscle fibers.

Of our 450 autopsies 118 disclosed inflammatory myositis. In 83 of these 118 cases (70.3 per cent) there was also one or more of the degenerative changes noted in the muscles. Degenerative processes were more common than inflammatory lesions. Degenerative muscular lesions (atrophy, cytoplasmic changes or nuclear changes) were noted in 256 of the 450 cases (56.8 per cent). One or more of the degenerative lesions or inflammatory nodular lesions were present in 293 of the 450 cases (65.1 per cent). While either inflammatory or degenerative processes may occur alone, there is definite overlapping. An extreme degree of inflammation was more commonly associated with degeneration than a lesser degree. There is a strong suggestion that the inflammation and degeneration may commonly result from the same cause.

COMMENT

An attempt has been made to evaluate the significance of nodular polymyositis and degenerative lesions (atrophy, cytoplasmic changes and nuclear variations) in 44 specimens of the deltoid muscle in cases of rheumatoid arthritis and in seven muscles from each of 450 routine autopsies.

The inflammatory lesions were much more common in the biopsy specimens of the deltoid muscle obtained in cases of rheumatoid arthritis than in the autopsy specimens of the same muscle. This tends to support the belief that this type of muscular lesion is part of the rheumatoid state. The lesions did not differ qualitatively in the two groups. The inflammatory lesions were characterized in the main by lymphocytic infiltration between muscle fibers and muscle bundles. The degenerative lesions were much more common in the cases coming to autopsy, but similar lesions, though fewer, were present in the biopsy material. Whether the nodular areas of myositis represent a characteristic or specific reaction to the possible infective agent of rheumatoid arthritis may be interpreted differently by different observers. Steiner considered the reaction a specific one for rheumatoid arthritis. It is significant that this type of inflammatory reaction was present in all our cases of rheumatic fever. This might be interpreted as suggesting a common relationship between acute rheumatic arthritis and rheumatoid arthritis. In our opinion this type of inflammatory reaction, while decidedly common in rheumatic and rheumatoid arthritis, is not a specific reaction. It was commonly found in muscles in cases of death due to accident or trauma and in many cases in which death was in no way correlated with any type of acute or chronic infection. On the other hand, rheumatoid arthritis to some extent is a common condition in Minnesota and may have been present to some degree without being mentioned in the histories.

There appears to be a definite but not an absolute relationship between the inflammatory lesions and the degenerative processes. They may occur separately, however. The degenerative processes are more commonly associated with a severe inflammation. On the basis of the degree of involvement the inflammation appears probably to be the first and primary lesion. It was noted that inflammation and degeneration were rarely found in the cases of acute poliomyelitis.

With the exception of the cases with rheumatic fever, inflammatory and degenerative types of lesions were more common in older people regardless of the type of disease causing death. The sexes were about equally affected.

Among the large variety of diseases in the autopsy cases there appeared to be no type of disease except acute rheumatic endocarditis in which the inflammatory or the degenerative lesions were more common.

In the single case of acute lupus erythematosus inflammatory nodules were found in six of the seven muscles. There was not a fibrinoid type of reaction in the connective tissue so commonly referred to as a specific reaction in lupus erythematosus.

The impression is gained from the observations in the 44 cases of rheumatoid arthritis and 450 autopsies with multiple causes of death, that muscular infection, atrophy, Zenker's degeneration and necrosis are common findings, and while most common in rheumatic and rheumatoid arthritis, these lesions are probably not the result of a specific infectious agent.

CONCLUSIONS

Nodular myositis and muscular degenerative lesions (atrophy, sarco-plastic and nuclear changes) are commonly found in cases of rheumatoid and acute rheumatic arthritis and to a lesser extent in an ordinary series of autopsies.

There appears to be some correlation between the inflammatory and degenerative lesions, but either may be present alone. The degenerative lesions occur more frequently than the inflammatory lesions.

Nodular myositis is more commonly seen in biopsies of deltoid muscles in cases of known rheumatoid arthritis than in postmortem studies of deltoid muscles.

The sexes are about equally involved.

No particular type of disease except rheumatic fever and rheumatoid arthritis seems to increase the frequency of the inflammatory and degenerative lesions.

The lesions are found more frequently in cases in which death occurred in the upper decades of life.

It is doubtful whether the lesions, on a morphologic basis, can be considered as a specific reaction to the infective agent of either acute rheumatic arthritis or rheumatoid arthritis, but the lesions probably are a part of the rheumatic and the rheumatoid state.

A SEARCH FOR CARCINOGENIC SUBSTANCES IN CARCINOMATOUS HUMAN LUNGS

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CARCINOMA of the human lung is unique among the common types of human visceral tumors in that in some cases its causes are known.¹ These causes account for only a small proportion of all pulmonary cancers. A number of agents after being inhaled into the lung are followed by cancer in such a conspicuous number of instances that a cause and effect relationship may reasonably be assumed. These substances may therefore be regarded as exogenous carcinogens. Among these inducers of pulmonic cancers may be mentioned: the ores of mines of Saxony² and Bohemia,³ whose active components may be radioactive substances⁴; asbestos⁵; possibly also chromium compounds,⁶ iron,⁷ nickel^{4a} and tar.⁸ A critical analysis of this problem is given by Hueper.^{4a}

Evidence that additional exogenous carcinogens producing pulmonary carcinoma might exist has come from studies of the occupational incidence of this neoplasm. Thus Kennaway and Kennaway⁹ found that in metal

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1. One outstanding exception is the urinary bladder tumor in workers with analine dyes probably induced by beta naphthylamine.

2. Arnstein, A.: *Verhandl. d. deutsch. path. Gesellsch.* **16**:332, 1913. Härtling, F. H., and Hesse, W.: *Vrtljschr. f. gerichtl. Med.* **30**:296, 1879; **31**:102, 1879.

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grinders the incidence of carcinoma of the lung was two and one-fourth times as high as it is in the general population. It was also high in occupations in which workers were exposed to road dust, coal gas and tar, and tobacco. Turner and Grace¹⁰ reported a significantly excessive mortality from cancer of the respiratory system in engineers, foundry workers and grinders.

Pulmonary tumors have been produced with carcinogenic compounds in experimental animals by many methods in numerous laboratories. The methods include direct application of carcinogens to lung tissue,¹¹ skin painting,¹² oral administration¹³ and subcutaneous,¹⁴ intratracheal,¹⁵ intravenous¹⁶ and intraperitoneal injection.¹⁷ Only a few of the methods are here mentioned. The experiments have in common exogenous carcinogens acting on cells of the lungs with resulting tumors.

Atmospheric dusts and substances which are at times found in such dusts have also been tested for their ability to induce tumors. Campbell, in a long series of studies,¹⁸ found a number of dusts and other substances of the environment to be carcinogenic for the lungs of mice. He stated, "There is no fundamental reason why the results obtained in mouse experiments with dusts should not be applied to man." Seelig and Benignus¹⁹ reported that mice exposed to coal smoke soot had an incidence of pulmonary tumors of 8 per cent, compared with 2 per cent in controls. Shimkin and Leiter²⁰ found a benzene-soluble substance in chimney soot that increased the number of pulmonary tumors in C3H mice. McDonald and Woodhouse²¹ caused mice to inhale atmospheric dusts and found that pulmonary tumors developed in 23 per cent, whereas 16 per cent of the controls had tumors. Leiter

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21. McDonald, S., Jr., and Woodhouse, D. L.: *J. Path. & Bact.* **54**:1, 1942.

and Shear²² reported that carcinogenic tars were extractable from air dusts of a number of American cities.

The principle is established that inhaled substances can act as carcinogens in man. It remains to be determined how completely the causation of cancers of human lungs is explained by carcinogens.

An attempt to demonstrate carcinogens in human lungs was reported by Kleinenberg, Neufach and Schabad.²³ They tested benzene extracts from 20 noncancerous persons and 19 cancerous persons, 2 of whom had primary carcinoma of the lungs. The extracts from cancerous persons were injected into 46 mice, which survived to 8 months of age or more. In a mouse which had received an extract of lung from a person who had cancer of the gallbladder, there developed at the site of injection a tumor, diagnosed as sarcoma. The incidence of tumors of other sites was greater in their experimental mice than in the controls. They expressed the belief that they had demonstrated a carcinogen and that the blastomogenic substance was endogenous.

In view of the evidence that certain inhaled substances cause pulmonary carcinoma in man, that additional unrecognized exogenous carcinogens possibly exist and that atmospheric dusts may contain substances that are carcinogenic, at least for animals, it seemed worth while to examine human lungs which contained primary carcinomas as well as control lungs for tumor-inducing substances. Such a study is herewith reported.

SELECTION OF CASES

Experiments were performed with 62 different extracts of 106 lungs from 101 different persons. Sixty of the extracts were made from the lungs of 55 adults; in 5 cases the two lungs were tested separately, because one of each pair contained a primary carcinoma.

These persons had lived in Chicago or the surrounding area. None showed clinical or postmortem evidence of industrial or occupational disease, except that some had excessive amounts of anthracosis. Their degree of exposure to hypothetical atmospheric carcinogens as indicated by their ages in decades was: second, 1; third, 3; fourth, 7; fifth, 6; sixth, 17; seventh, 14; eighth, 6; ninth, 1. Thirty-eight were over 50 years old. Thirty-five were males, and 20 were females. These people probably had been exposed to atmospheric dusts to the degrees average for inhabitants of this community.

The lungs of 46 stillborn infants, because of their small size, were pooled into two specimens (I and II) for extraction and testing. Insufficient material was extracted from the lungs of individual infants to enable a satisfactory biologic test to be performed (average was 0.32 Gm.). Some of these infants showed various stages of prematurity but they had in common the fact that they had not inhaled any air.

The lungs may be divided into five groups:

22. Leiter, J., and Shear, M. J.: *J. Nat. Cancer Inst.* 3:167, 1942.

23. Kleinenberg, H. E.; Neufach, S. A., and Schabad, L. M.: *Cancer Research* 1:853, 1941.

Group A. Lungs Containing Primary Carcinoma.—This group consisted of 12 lungs or pairs of lungs containing a primary cancer.

Group B. Noncancerous Lungs Contralateral to Lungs That Contained Primary Carcinoma.—The nontumorous lung opposite the lung containing a primary carcinoma was examined in 7 cases. If pulmonary cancers are caused by inhaled carcinogens which accumulate slowly and produce tumors only after a relatively long period of induction, the lungs opposite cancerous lungs should also contain the carcinogens, at least in some cases.

Group C. Lungs from Persons Who Had No Cancers.—The 23 lungs composing this group were tested for four reasons: (a) They might contain inhaled carcinogens which had not yet induced a tumor. (b) They might contain a carcinogen, possibly endogenous, corresponding to that found in human liver.^{24b} (c) They serve as one type of control for the methods of extraction, yielding information on whether tissue carcinogens are chemical conversion products. (d) They add an important organ to those previously studied in a survey of the human body for carcinogens.²⁵ These persons died from causes commonly encountered in the necropsy service of a general hospital. A list of the diseases represented is given in table 1.

Group D. Lungs from Persons Who Had a Cancer Primary Elsewhere in the Body.—Several of the 18 persons whose lungs composed this group had small pulmonary metastases of tumor, but the majority of the lungs were tumor free. These lungs were tested for the same reasons as were those in group C. In addition, it was desirable to know whether a hypothetical carcinogen transported from a cancer primary elsewhere in the body became localized in the lungs. The types of primary tumors are given in table 1.

Group E. Lungs from Stillborn Infants.—These infants had not inhaled air and their lungs could contain no atmospheric carcinogen. If the extracts showed tumor-inducing activity, the substance would, of necessity, be either an endogenous carcinogen, a chemical conversion product or a carcinogen transmitted from the mother. From other experiments it is known that extracts of the livers of some of these same infants exhibited carcinogenic activity.²⁵

PREPARATION OF THE EXTRACTS

The fresh lungs were finely divided with knife or grinder and preserved in 1 volume of 95 per cent ethanol. (Exception: Three lungs were preserved in Kaiserling solution.) Extracts were made by the method previously described.^{24a} Essentially it consisted of alkaline hydrolysis and ethylene dichloride extraction of the nonsaponifiable lipids. Saponification was repeated once on the extract so obtained. The final extracts were pale tan or yellow to brown pasty or flaky materials with a pungent odor.

The weight of lung tissue, the amount of extract and the percentage of extract are given in table 1. There were great individual differences in the weights of the lungs because in some cases not all of the lung tissue was available for extraction and because there were great differences in the amount of pathologic involvement present, such as edema, hyperemia, pneumonia and tumor. All the specimens included in group B were single lungs as were the 5 in group A and

24. Steiner, P. E.: (a) *Cancer Research* 2:425, 1942; (b) 3:385, 1943.

25. Steiner, P. E.; Stanger, D. W., and Bolyard, M. N.: *Cancer Research*, to be published.

TABLE 1.—*Nonsaponifiable Lipids Extracted from Human Lungs*

Designation of Case, Specimen and Extract	Major Diagnosis	Weight of Lung, Gm.	Weight of Extract, Gm.	Percentage of Nonsaponifiable Lipids
Group A. Lungs containing primary carcinoma:				
5182	Carcinoma of lung.....	1,953	13.2	0.68
5325	Carcinoma of lung.....	1,800	9.3	0.52
5458	Carcinoma of lung.....	436	3.5	0.80
5466	Carcinoma of lung.....	459	4.0	0.87
5470	Carcinoma of lung.....	1,811	5.1	0.28
5506	Carcinoma of lung.....	925	5.6	0.57
5533	Carcinoma of lung.....	873	4.4	0.50
5550	Carcinoma of lung.....	968	5.0	0.52
5564	Carcinoma of lung.....	810	5.7	0.70
5627	Carcinoma of lung.....	887	3.4	0.39
5632	Carcinoma of lung.....	744	2.4	0.32
5685	Carcinoma of lung.....	821	2.7	0.33
Group B. Noncancerous lungs contralateral to lungs that contained primary carcinoma:				
5355	Carcinoma of lung.....	291	1.6	0.55
5458	Carcinoma of lung.....	309	3.4	1.10
5466	Carcinoma of lung.....	341	2.5	0.73
5550	Carcinoma of lung.....	285	2.3	0.81
5564*	Carcinoma of lung.....	563	1.7	0.30
5575	Carcinoma of lung.....	480	2.4	0.50
5682	Carcinoma of lung.....	629	2.2	0.35
Group C. Lungs from noneancerous persons:				
5368	Pulmonary tuberculosis.....	1,130	3.3	0.29
5519	Malignant nephrosclerosis.....	750	5.6	0.75
5521	Pyelonephritis and diabetes.....	327	2.2	0.67
5522	Coronary thrombosis.....	730	3.8	0.52
5525	Nonspecific ulcerative colitis.....	528	1.5	0.28
5527	Nonspecific ulcerative colitis.....	783	2.8	0.36
5528	Abscess of the lung.....	638	2.8	0.31
5531	Streptococcal pyemia.....	1,014	3.8	0.37
5542	Fracture of femur.....	355	5.6	1.58
5549	Rheumatic heart disease.....	750	2.7	0.36
5581	Hypertensive cardiovascular disease.....	986	5.8	0.62
5603	Rheumatic heart disease.....	435	1.9	0.44
5607	Streptococcal septicemia.....	275	1.2	0.44
5610	Rheumatic heart disease.....	387	2.1	0.54
5623	Pulmonary embolism.....	775	3.0	0.39
5636	Bile peritonitis.....	625	2.4	0.38
5638	Arteriosclerotic heart disease.....	1,230	3.7	0.30
5639	Bacterial endocarditis.....	360	1.3	0.36
5644	Glaucoma.....	472	1.5	0.32
5645*	Rapidly progressive hypertension.....	1,080	5.9	0.55
5648	Encephalomyelitis.....	820	2.2	0.27
5657	Malignant nephrosclerosis.....	860	5.6	0.65
5661	Congenital renal agenesis.....	345	1.6	0.46
Group D. Lungs from persons with nonpulmonic cancers:				
5544	Carcinoma of stomach.....	395	2.1	0.53
5571	Carcinoma of stomach.....	1,750	6.9	0.39
5599	Carcinoma of stomach.....	341	1.3	0.38
5655	Carcinoma of stomach.....	1,170	3.7	0.32
5578	Leukemia.....	620	3.0	0.48
5612	Leukemia.....	799	4.4	0.56
5617	Leukemia.....	840	2.9	0.34
5541	Carcinoma of colon.....	732	1.8	0.25
5614	Carcinoma of colon.....	424	2.3	0.54
Eng. 41	Carcinoma of kidney.....	1,215	8.7	0.72
5532	Carcinoma of kidney.....	751	3.3	0.44
5667*	Carcinoma of prostate.....	795	2.6	0.33
5680	Carcinoma of prostate.....	793	2.0	0.25
5529	Carcinoma of breast.....	308	1.6	0.52
5569	Carcinoma of uterus.....	990	3.4	0.34
5653	Carcinoma of liver.....	1,180	5.0	0.42
5658	Carcinoma of bile ducts.....	1,150	3.8	0.33
5656	Lymphoblastoma.....	920	3.9	0.42
Group E. Lungs from stillborn infants:				
I	(26 pairs of lungs).....	1,003	6.7	0.07
II*	(20 pairs of lungs).....	1,049	5.0	0.76

* These extracts later proved to be carcinogenic.

scattered specimens in the other groups. The extracts also varied greatly in amount because they represented the nonsaponifiable lipids not only of the original pulmonary tissue but also of any tumor, inflammatory exudate and other solid component which was present. The extracts expressed in percentage of weight of original tissue also show great variation, partly at least because there were great differences in the amount of pulmonary edema and other fluids. These factors combine to hide any possible differences in the amount of extract attributable to inhalation of carcinogenic materials. This statement is true regardless of whether the comparison is by individual cases (table 1) or by groups (table 2).

The extracts were mixed with 3 volumes of tricaprylin for injection.

TESTING THE EXTRACTS

The extracts were tested for carcinogenic activity as follows: Mice were used. Each animal was given by subcutaneous injection a total of about 500 mg. in 1.5 cc. of tricaprylin. This amount was administered in two doses given four weeks apart. The number of mice receiving injections in each experiment varied from 3 to 27, according to the amount of extract available. The mice were about

TABLE 2.—*Summary of Extractions of Nonsaponifiable Lipids of Human Lungs*

Experimental Group	Persons from Whom Lungs Were Obtained	Average Weight of Lungs, Gm.	Average Weight of Extract, Gm.	Percentage of Nonsaponifiable Lipid Extract
A.....	12	1088.9	5.36	0.52
B.....	7	414.0	2.80	0.56
C.....	23	678.4	3.14	0.47
D.....	18	844.1	3.43	0.41
E.....	46	44.6	0.32	0.71

equally divided as to sex. They were of two strains. Thirty-seven extracts were tested in 315 mice of the C57 Black strain. These mice were from 2½ to 6½ months old. Some were obtained from the Roscoe B. Jackson Memorial Laboratory and some were raised in our laboratory as the first generation offspring of breeding stock obtained from Bar Harbor. Twenty-five extracts were injected into 129 mice of our own partly inbred albino strain.²⁶ These mice were from 2 to 5 months old.

The extracts of infant lungs were toxic in doses of 250 mg. dispersed in 0.75 cc. of tricaprylin. Consequently they were administered at the rate of 125 mg. in 0.37 cc. of tricaprylin at intervals of four weeks. The mice used for the group E I extract received a total of only 375 mg. of extract, but those used for the group E II extract had the usual 500 mg.

The mice were fed a diet previously described.^{24b} They were examined at frequent intervals. Necropsies were performed on all animals, and all lesions suggestive of neoplasm were examined microscopically. The experiments were terminated early in the twenty-fifth month.

Control experiments were performed in which 154 mice of the C57 Black strain were given by subcutaneous injection 2 cc. of tricaprylin alone. This control was made because the diet and possibly other environmental conditions of this laboratory are different from those at Bar Harbor.

26. Steiner and others.²⁵ Steiner.²⁴

RESULTS

One lung extract (5525), obtained in a case of nonspecific ulcerative colitis, exhibited immediately after injection the high toxicity previously observed in cases of this disease²⁷ and noted occasionally to a lesser degree with other human tissue extracts.²⁶ It killed all mice, and consequently it was not tested for carcinogenicity. The extracts of both the pooled specimens of infant lungs also were toxic, but tests were successfully made by injecting them in four doses.

Some extracts were caustic to the tissues and sloughed out wholly or in part. This phenomenon has previously been observed with extracts of human liver, spleen, colon and other materials.²⁶ It is again mentioned because it prevented quantitative determinations of carcinogenic potency. This necrotizing factor was not recognizably related to the major disease diagnosed or to the subsequent carcinogenicity of the extract. The estimated losses of the extract in percentage were as follows: 0, 3 cases; 1 to 24, 23 cases; 25 to 49, 14 cases; 50 to 74, 12 cases; 75 to 100, 9 cases.

Survival of the mice was satisfactory in most experiments. Of the 474 originally receiving injections, 413 survived for six months, 355 for twelve months, 220 for eighteen months, and 74 were alive at the beginning of the twenty-fifth month after injection, when the experiments were terminated.

Six sarcomas were found at sites of injections of four extracts, of which three were from adult lungs and one from lungs of infants. The cases are abstracted as follows:

5564. The nontumorous lung from which the extract was obtained and which was the mate of a lung containing primary carcinoma was that of a railroad brakeman 56 years old. It weighed 563 Gm. and yielded 1.7 Gm. of extract, which was injected into 4 C57 Black male mice. One mouse died in the thirteenth month with a large mixed spindle and polymorphous cell sarcoma at the site of injection. Another mouse died in the eighteenth month with a similar tumor. One died in the tenth month with a reticulum cell sarcoma involving the liver, the spleen and the lymph nodes. The last animal died in the twenty-third month without tumor.

5645. The extract (5.9 Gm.) was obtained from the lungs of a business man 56 years old who died of rapidly progressive hypertension. It was tested in 12 albino strain male mice. One died in the eighteenth month of a large spindle cell carcinoma of the injection site, leaving 4 survivors, 2 of which subsequently succumbed to pulmonary tumors in the twenty-first and twenty-fourth months.

5667. The extract (2.6 Gm.) was obtained from the lungs of a decorator aged 62 years who had a carcinoma of the prostate. It was tested in 5 albino strain male mice. One spindle cell sarcoma was found at the site of injection in the twenty-fourth month. At the same time 2 mice had pulmonary tumors.

27. Steiner, P. E.; Stanger, D. W., and Bolyard, M.: *Proc. Soc. Exper. Biol. & Med.* 55:8, 1944.

Stillborn Infant Pooled Speciment II. The extract (8.0 Gm.) was injected into 16 albino strain female mice, of which 11 were alive at twelve months, 8 at eighteen months and 1 at twenty-four months after injection. Mice died with spindle cell sarcomas at the injection sites in the twenty-first and twenty-fourth months. In addition, 4 animals died of leukemic disease in the eighteenth, twenty-first, twenty-second and twenty-third months; 1, of pulmonary tumor in the seventeenth month, and 1, of mammary gland carcinoma in the twenty-fourth month.

In table 3 the results are summarized with respect to the sarcomas at sites of injection. The percentage yield (calculated on the basis of the number of mice living at the time when the first sarcoma appeared in each experiment) varied from 20.0 to 66.7, with an average of 33.3.

TABLE 3.—*Sarcomagenic Activity of Nonsaponifiable Lipids Extracted from Human Lungs*

Experi- mental Group	Source of Extracts	Ex- tracts Tested	Mice Used	Mice Alive at 6 Mo.	Effective Total* Num- ber of Sar- coma- Mice in genic Which (Ac- Ex- tracts tive) Were In- duced			Per- centage Yield with Active Extracts
					6 Mo.	Ex- tracts	Sar- coma- Mice in genic Which (Ac- Ex- tracts tive) Were In- duced	
A	Lungs with primary carcinoma.....	12	129	115	0	0	0	0
B	Noncancerous lungs contralateral to lungs with primary carcinoma.....	7	33	31	1	3	2	66.7
C	Lungs from noncancerous persons..	23	146	131	1	5	1	20.0
D	Lungs from persons with cancer other than pulmonic.....	18	127	119	1	3	1	33.3
E	Lungs of stillborn infants (pooled)..	2	39	12	1	7	2	29.9

* The number of mice living when the first tumor appeared is used as the "effective total" number.

Summaries of experiments in which tumors occurred or did not occur are given in table 4, together with other pertinent data. The experiments are divided into three sections: (a) those experiments in which sarcomas were found at sites of injection, (b) those in which no sarcomas occurred and (c) tricapyrylin controls. This arrangement is used to facilitate the analysis of tumors which occurred at distant sites.

The subcutaneous spindle and polymorphous cell sarcomas which were found at sites of injection can safely be regarded as induced tumors inasmuch as such neoplasms do not develop spontaneously in either of the strains of mice used. Whether they were induced by a sarcogenic component of the extracts might be challenged, because 1 control mouse, given tricapyrylin alone, had a similar tumor. The differences in the percentage yield of sarcomas (calculated on the basis of the number of tumors in the mice surviving the injection six months) are, however, probably significant in view of the number of mice tested (18.8 per cent versus 0.7 per cent). The extracts are regarded as having shown carcinogenic activity.

TABLE 4.—Summaries of Experiments in Which Tumors Were or Were Not Produced with Extracts of Human Lungs

Designation of Case, Specimen and Extract	Mice Receiving Injections		Mice Surviving Given Number of Months						Sarcomas at Sites of Injection		All Other Tumors										
			Strain	Sex	No.	6 Mo.	12 Mo.	18 Mo.	24 Mo.	%*	No.	%	Lung		Lymphatic		Mammary Gland		Miscellaneous		
	No.	%											No.	%	No.	%	No.	%			
Experiments in which sarcomas developed at sites of injection:																					
5364 (nonneurotic).....	C57 Black	Male	4	4	3	1	0	2	50.0	0	0.0	0	0.0	1	25.0	0	0.0	0	0.0	0	0.0
5645.....	Albino	Male	12	12	10	4	0	1	8.3	2	16.6	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
5667.....	Albino	Male	5	5	5	4	3	1	20.0	2	40.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Infant lungs, II.....	Albino	Female	16	11	11	8	1	2	18.2	1	9.1	4	36.4	1	9.1	0	0.0	0	0.0	0	0.0
Totals.....	37	32	29	17	4	6	18.8	5	15.6	5	15.6	1	3.0	0	0.0	0	0.0	0	0.0
Experiments in which no sarcomas developed at sites of injection:																					
36 combined cases.....	C57 Black	Both	311	265	246	169	62	0	0.0	6	2.3	32	12.1	0	0.0	10	3.8	1	0.9	0	0.0
22 combined cases.....	Albino	Both	126	116	96	46	12	0	0.0	18	15.5	4	3.5	4	3.5	1	0.9	0	0.0	0	0.0
Totals (58 cases).....	437	381	342	215	74	0	0.0	24	6.3	36	9.6	4	1.1	11	3.0	0	0.0	0	0.0
Tricaprilyn controls:																					
.....	C57 Black	Both	154	149	116	72	0	1	0.7	2	1.4	10	6.7	0	0.0	0	0.0	0	0.0	0	0.0

* In this table the percentage yield is calculated from the number of tumors in six month survivors.

The tumors found elsewhere in the mice—the lungs, the lymphatic system, the mammary glands and other structures—are also given in table 4. Tumors of distant sites can be induced by subcutaneous injections of carcinogens. If the incidence of such tumors is significantly increased above normal for the strain by the injection of substances of unknown carcinogenic activity, it may be concluded that the material is carcinogenic. In the present analysis the incidence of tumors of the lungs appears greater in the combined experiments in which the extracts were locally sarcomagenic than in those in which they were not, or in the tricaprylin controls. However, most of the increase was contributed by albino strain mice. When the figures for the incidence of pulmonary tumors in the two groups of experiments are compared for each strain separately, no increase is found. A small increase in lymphatic tumors (12.1 versus 6.7 per cent) was found in C57 Black mice given injections of lung extracts when these mice were compared with tricaprylin controls. This constitutes the only acceptable evidence for an increase of tumors of distant sites.

COMMENT

Evidence that a carcinogenic factor was present in extracts of some human lungs was obtained in these experiments. The extracts induced sarcomas at sites of their injection, and they probably increased the incidence of lymphatic tumors in C57 Black mice.

Evidence was also obtained that the active factor was endogenous rather than exogenous; it was already present in the lungs of stillborn infants who had not inhaled air, although some of them probably had aspirated amniotic fluid. Transplacental transmission of an exogenous factor was not eliminated as a possibility, but it seems highly remote because of the failure to detect activity in the majority of extracts of adult lungs. In other experiments extracts of the livers of some of these same infants were carcinogenic, pointing again to an endogenous factor.

Failure to demonstrate exogenous carcinogens in these experiments does not prove their absence. Only one method of extraction was used, and only one type of extract was tested. Furthermore, it should be noted that none of the carcinomas of the lungs which were tested had been induced by any of the recognized or strongly suspected exogenous pulmonary carcinogens. Neither did the lungs exhibit any of the industrial or occupational diseases which sometimes accompany or terminate in cancer. Studies of such lungs by this and other methods might prove profitable.

These experiments revealed no causative exogenous factor for pulmonary cancer. The three extracts of adult lungs which were sarcomagenic came from the control groups. One extract (5564) which

induced 2 tumors in 4 mice was made from a noncancerous lung whose mate contained a primary carcinoma and yielded an inactive extract. The 3 adult lungs which gave sarcomagenic extracts did not have any pulmonic disease in common. Two factors were shared by all 3: (a) They were all from men. (b) These men all had disease of the prostate; one had primary carcinoma of the prostate gland, and the others had benign adenomatous hyperplasia despite their age, 56 years, which is below the usual age for this condition. The possible relationship of sarcomagenic activity of human tissue extracts and hormonal imbalances has previously been mentioned.^{24b} Its significance, if any, in the present instance is not known.

Human lung may be added to liver and spleen as examples of tissues which in a survey of the body have yielded sarcomagenic extracts.²⁵

It is known from studies of carcinogens that minute quantities may induce tumors in animals. Shear²⁸ reported that 0.0004 mg. of 1, 2, 5, 6-dibenzanthracene induced a sarcoma in a mouse. It is possible that small quantities of a carcinogen may induce tumors in man and that in the process the chemical is changed to a new form. It might be difficult or impossible to recover the carcinogen from such tumors. For these reasons failure to demonstrate carcinogens does not prove their absence. If a carcinogen were obtained from a cancerous lung, it might represent merely an excess, an unutilized residue, and not that portion which actually induced the tumor.

Four of 62 extracts that were tested induced sarcomas. This result is not to be interpreted as an absolute and final measure of the degree of carcinogenicity of lung extracts, any more than were the results of testing extracts of individual livers.^{24b} There appears to be a definite "threshold" phenomenon in the testing of these crude tissue extracts. It is probable that both the number of extracts which induced tumors and the number of tumors which they induced would have been increased if any or all of the following conditions had prevailed: (a) If after injection the material had been better retained. (b) If the mice had lived longer. (c) If mice more highly susceptible to sarcoma had been used. The results obtained pertain only to the experiments performed. Better methods may give different results in the future.

SUMMARY

The nonsaponifiable lipid extracts of 106 human lungs were tested for carcinogenic activity in 62 separate experiments in which 474 mice of C 57 Black and our albino strains were used. The lungs were those containing primary carcinoma; noncancerous lungs contralateral to those containing primary carcinoma; lungs of persons free from cancers;

28. Shear, M. J.: *Am. J. Cancer* 26:322, 1936.

lungs of persons with cancer primary elsewhere in the body, and lungs of stillborn infants.

Six sarcomas were induced at sites of injection of four different extracts. This constitutes a 33.3 per cent yield if the yield is calculated on the basis of the number of mice living when the first tumor appeared in each of the experiments with active extracts. The percentage yield calculated on the basis of the number of tumors in six month survivors was 18.8. In addition, the incidence of lymphatic tumors in C 57 Black mice was possibly increased above normal.

The sarcomagenic extracts were derived, respectively, from a non-tumorous lung opposite a lung with primary carcinoma, the noncancerous lungs of a person with carcinoma of the prostate, the lungs of a person with rapidly progressing hypertension, and the pooled lungs of stillborn infants.

The sarcoma-inducing activity of an extract of lungs of infants who had not inhaled air indicates that the sarcomagen is probably endogenous. Human lung may be added to liver and spleen as examples of tissues from which extracts with tumor-inducing activity have been obtained.

NONSPECIFIC MYOCARDITIS

Analysis of a Series of Thirty-Six Cases

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THE PROBLEM of myocarditis has received increasing attention in recent years. Most of this interest has been confined to the isolated form which is also known as Fiedler's myocarditis. The importance of this disease is illustrated by the fact that Moritz and Zamcheck¹ found 14 cases of isolated myocarditis among "between 200 and 300" instances of unexpected death in young soldiers.

In most of the reported cases of Fiedler's myocarditis the leukocytic infiltration was of a diffuse type. There is, however, evidence suggesting that the anatomically less conspicuous focal type of inflammation may also be of considerable importance. Saphir,² reporting 5 cases of myocarditis combined with laryngeal edema in children, stated that the areas of inflammation were widely scattered throughout the myocardium and that in 2 instances ten blocks had to be sectioned before such lesions were found. Clinical observations further add to the concept that focal myocarditis is not infrequent and that it may be of practical import. Glatthaar³ expressed the belief that foci of inflammation around vital branches of the coronary arteries or within the conductive system may be responsible for cardiac symptoms as well as for electrocardiographic changes and occasionally also for sudden death. Scherf⁴ found that clinical evidence of myocardial involvement developed in 10 to 15 per cent of patients with acute tonsillitis. Scherf and Boyd⁵ go as far as to imply that in view of the frequency of infectious diseases and localized infections there are but few persons who at some time or another do not have small inflammatory myocardial foci. Candel and Wheelock⁶ arrived at a similar opinion from their observations in 11 cases of acute nonspecific myocarditis.

From the Department of Pathology, Baylor University College of Medicine.

1. Moritz, A. R., and Zamcheck, N.: *Arch. Path.* **42**:459, 1946.

2. Saphir, O.: *Am. J. M. Sc.* **210**:296, 1945.

3. Glatthaar, D.: *Schweiz. med. Wchnschr.* **76**:74, 1946.

4. Scherf, D.: *Bull. New York M. Coll., Flower & Fifth Ave. Hosp.* **3**: 252, 1940.

5. Scherf, D., and Boyd, L. J.: *Cardiovascular Diseases*, St. Louis, C. V. Mosby Company, 1939, pp. 176-180.

6. Candel, S., and Wheelock, M. C.: *Ann. Int. Med.* **23**:309, 1945.

It appears therefore justified to include instances of focal myocarditis in the series presented here.

SELECTION OF CASES

The group comprises 36 cases that were collected from a total of 3,800 autopsies. The diagnosis was based on the microscopic examination of routine sections. In the large majority of cases the blocks were taken from the wall of the left ventricle or from the interventricular septum. On the average, two blocks were cut in each case. In general, only those instances were included in which there was either extensive diffuse leukocytic infiltration or unequivocal evidence of focal inflammation. However, for reasons explained later, 4 cases with minimal focal inflammatory involvement were added. Cases in which any of the following findings were made at autopsy were not included in this series: rheumatic heart disease, myocardial changes due to lesions of the coronary arteries, extensive myocardial fibrosis, bacterial endocarditis, acute pericarditis, pyemic abscesses of the myocardium and specific granulomas. Also excluded were all cases of specific infections that are known to cause myocardial lesions, such as diphtheria and scarlet fever.

It is obvious that difficulties could arise in ruling out rheumatic lesions. All cases were excluded in which a history of rheumatic fever was given or in which Aschoff bodies, epicardial or endocardial involvement unless of a minimal degree, fibrinoid swelling or perivascular fibrosis was shown. Syphilis as a cause was eliminated as far as possible by omitting all cases in which a history of syphilitic infection was given or in which serologic tests were positive, as well as those in which the autopsy findings were indicative of syphilis. Results of serologic tests were available in only about one half of the cases, mainly because many of the patients died after having been in the hospital for only a short time.

Cases of chronic myocarditis with formation of giant cells and granulation tissue, such as the case of "myocarditis perniciosa" described by Boikan,⁷ were not added to this series since it appeared hardly possible to differentiate the lesions from specific granulomas.

PRESENTATION OF FINDINGS

Age.—The ages of the patients ranged from 1 month to 86 years. Twenty-two of the patients were below the age of 40, and 8 of these were less than 15 years old.

Sex.—The group included 26 males and 10 females. The percentage of males in the entire autopsy series was 65.

7. Boikan, W. S.: *Virchows Arch. f. path. Anat.* **282**:46, 1931.

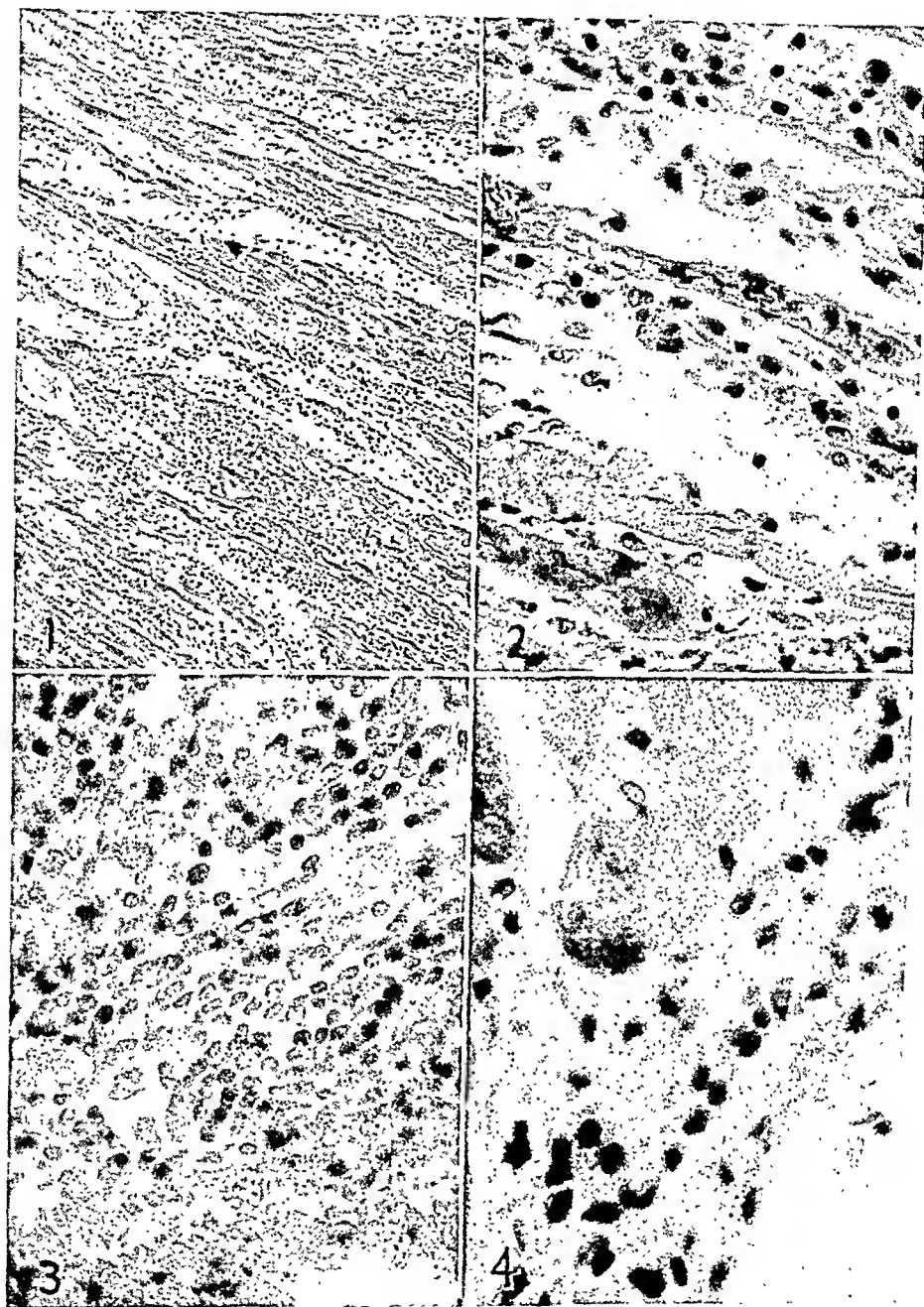


Fig. 1.—Diffuse myocarditis; $\times 65$. The infiltration is most prominent in the interstitial tissue.

Fig. 2.—Diffuse myocarditis; $\times 250$. Infiltration of an interstitial space is shown, with involvement of the adjacent muscle fibers.

Fig. 3.—Focal myocarditis; $\times 250$. A large focus is destroying muscle fibers immediately adjacent to an interstitial blood vessel.

Fig. 4.—Focal myocarditis; $\times 450$. This is a perivascular lesion containing elongated cells with distorted nuclei.

Gross Findings.—These were, in general, confined to cardiac hypertrophy, which was noted in 15 cases. Only in a few instances a gray discoloration of the myocardium was seen. In over half of the cases gross changes were absent.

Microscopic Changes.—The basic lesion was a leukocytic infiltration, which was always of interstitial nature, frequently showing a tendency toward perivascular arrangement (fig. 1). In many cases there were, in addition, infiltration and partial destruction of some of the myocardial fibers. The parenchymal changes seemed to be of a secondary nature since, with rare exceptions, the lesions in the fibers could be traced to interstitial infiltrates in adjacent areas (figs. 2 and 3).

The cellular composition is analyzed in table 1. The only constant finding was the presence of elongated cells with large, distorted nuclei. Although cells of this type were never predominant, they were found to be present in most instances.

TABLE 1.—*Distribution and Composition of Myocardial Infiltrates*

Distribution	Cases in Which the Predominant Cells Were			Cases in Which No Type of Cell was Predominant	Cases in Which Eosinophils Were Present
	Polymorpho-nuclear Leukocytes	Plasma Cells	Lymphocytes		
Diffuse.....	5	4	3
Focal.....	10	2	8	..	3
Focal and minimal	4	..

The type cell has a compact, dark-staining nucleus, which is often curved or distorted and which averages 10 microns in length. The cytoplasm is scanty and indistinctly outlined, is faintly acidophilic and forms short tapering processes at the poles of the cell (figs. 4 and 5).

These cells are morphologically different from the "myocyte" of Anitschkow and from the Aschoff cell. Their distribution precludes an origin from degenerating muscle fibers and makes it likely that they represent proliferating fibroblasts or endothelial cells. They appear to be related to the mesenchymal cells that are seen in rheumatic lesions and that might be the forerunners of the multinucleated giant cells.

Extracardiac Foci of Inflammation.—Inflammatory lesions in organs other than the heart could be found in 35 of the 36 cases. The distribution of the foci, as well as the character of the pulmonary lesions, are given in table 2. The extrapulmonary lesions were distributed among a wide variety of organs or structures (skin, liver, kidneys, peritoneum, prostate, gallbladder, brain and meninges).

Clinical Evidence of Cardiac Involvement.—Objective signs were present in 9 cases. Tachycardia was noted in 5 instances, cardiac enlargement in 2, arrhythmia in 1 and murmurs in 1.

Evidence of Drug Sensitization.—The application of sulfonamide compounds was recorded in 6 cases and could be definitely ruled out in 17 others. In the remaining 13 cases the role played by sulfonamide drugs could not be determined with certainty, especially since some of the patients had had treatment before entering the hospital. Only 1 instance of treatment with neoarsphenamine was found.

COMMENT

In this series nonspecific myocarditis was found in about 1 per cent of autopsies. Since the lesions may be small and widely separated, it appears likely that they remained unnoticed in some instances and that the true incidence is, therefore, higher than 1 per cent.

Considerable difficulty was encountered in deciding which of the cases should be designated as instances of "isolated" myocarditis. According to Saphir⁸:

. . . one is justified in accepting the occurrence of isolated myocarditis in the sense of a more or less diffuse inflammatory lesion if every known cause for this type of myocarditis is ruled out and if the myocarditis is found in the absence of any major pathologic condition involving either the endocardium and pericardium or the entire body.

TABLE 2.—*Site and Classification of Extracardiac Lesions*

Site	Cases
Lungs only.....	18
Lungs and other organs.....	8
Other organs only.....	9
Classification of Pulmonary Lesions	
Bronchopneumonia.....	8
Interstitial pneumonitis.....	8
Tuberculosis.....	4
Septic infarcts.....	1
Pleuritis.....	2
Lipid pneumonia.....	1
Bronchiectasis.....	2

In none of the cases presented here was any endocardial or pericardial involvement shown; but, with regard to lesions of other organs, it was difficult to decide which of these had to be called major pathologic conditions. Ten of the 12 cases with diffuse leukocytic infiltration of the myocardium should meet the requirements established for the isolated type. In 9 of them extracardiac foci were demonstrated only in the lungs, and none of these lesions were extensive; in the tenth there was evidence of chronic prostatitis.

It appears doubtful, however, whether a sharp line can be drawn between focal and diffuse involvement as well as between isolated and

8. Saphir, O.: Arch. Path. 32:1000, 1941.

complicating myocarditis. It seems more likely that the diffuse inflammation is merely an advanced stage due to confluence of disseminated lesions and that, as in the other types of myocarditis, inflammatory foci are found elsewhere in the body.

The question whether or not myocarditis is primarily of interstitial nature has been the occasion of considerable argument. Some authors present evidence that the process begins with damage of the myocardial fibers (Hansmann and Schenken⁹; Covey¹⁰). In the series of cases reported here, the inflammatory process seemed to have originated in the interstitial connective tissue, and any involvement of the muscle fibers was apparently of secondary nature. This impression was enhanced by examination of the group classified as "minimal myocarditis," in which the lesions were found exclusively within the

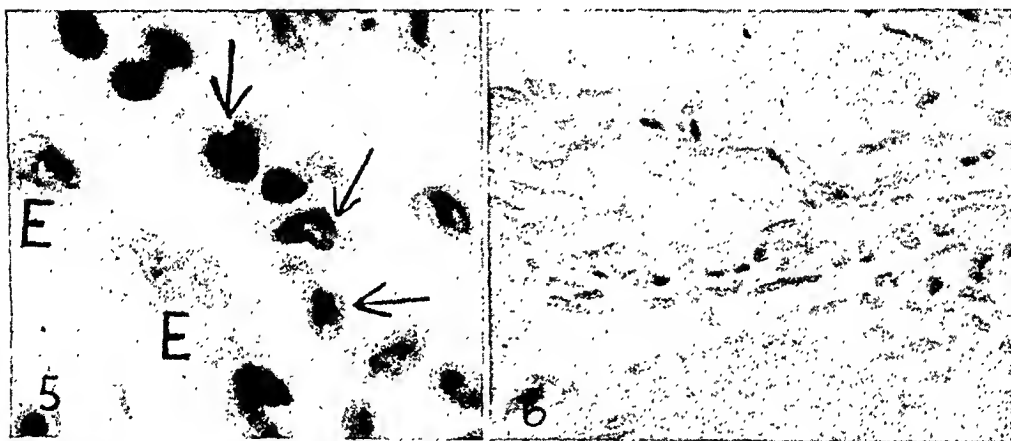


Fig. 5.—Part of the area shown in figure 4; $\times 950$. Arrows point to typical elongated cells with distorted nuclei. Cells marked *E* belong to the endothelium of a capillary.

Fig. 6.—Focal myocarditis; $\times 455$. This is a minimal focus in close vicinity to a capillary.

fibrous tissue septums. The 4 cases of minimal myocarditis were included in this series since, although the foci were minute, the same characteristic histologic picture was shown in all of them. The lesions were confined to the interstitial tissue, the myocardial fibers being intact in all 4 cases. The cellular elements consisted of small round cells, plasma cells and mesenchymal cells with distorted nuclei. Occasionally a small number of polymorphonuclear leukocytes were present. Increased vascularization was constantly found in the form of dilated thin-walled capillaries. In many instances the lesions were located close to interstitial arterioles.

9. Hansmann, G. H., and Schenken, J. R.: *Am. Heart J.* **15**:749, 1938.

10. Covey, G. W.: *Am. J. Clin. Path.* **12**:160, 1942.

Since minimal foci were occasionally found in the other cases, together with more extensive areas of inflammation (fig. 6), it appears likely that in the 4 instances of minimal myocarditis the process represents early stages of more severe involvement.

Although no cases were included in which any of the accepted anatomic stigmas of rheumatic disease were shown, there is no way to

TABLE 3.—*Review of Possible Causes*

Cause	Author	Nature of Evidence		
		Clinical	Post-mortem	Experimental
Bacterial infections.....	Scherf ⁴	+
	Schenken and Heibner ²⁰	+	..
	Glatthaar ³	+
Virus.....	Pearce, J. M.: Arch. Path. 34:319, 1942	+
	Covey ¹⁵	+	+	..
	Finland and co-workers ¹⁴	+	..
	Helwig, P. O., and Schmidt, E. O. H.: Science 102:31, 1945.....	+
Sensitization to various substances	Aplitz, K.: Virchows Arch. f. path. Anat. 289:46, 1933.....	+
	Clark E., and Kaplan, B. I.: Arch. Path. 24:458, 1937.....	..	+	..
	Brown, O. E., and McNamara, D. H.: Arch. Dermat. & Syph. 42:312, 1940	..	+	..
	Rich, A. R.: Bull. Johns Hopkins Hosp. 71:123, 1942.....	..	+	..
	French, A. J., and Weller, O. V.: Am. J. Path. 18:109, 1942.....	..	+	+
	Chafee, F. H.; Ross, J. R., and Gunn, E. M.: Ann. Int. Med. 17:45, 1942...	..	+	..
Drugs causing increased heart action	Fleischer, M. S., and Loeb, L.: Arch. Int. Med. 6:427, 1910.....	+
	Hueper, W. C., and Ichniowski, C. T.: J. Lab. & Clin. Med. 26:1565, 1941..	+
Dietary deficiency				
General malnutrition	Toreson, W. E.: Arch. Int. Med. 73:375, 1944	+	..
Potassium deficiency	Schrader, G. A.; Prickett, O. O., and Salmon, W. D.: J. Nutrition 14:85, 1937	+
	Follis, R. H.; Orent-Kelles, E., and McCollum, E. V.: Am. J. Path. 18:29, 1942	+
Combined deficiency of potassium and vitamin B	Thomas, R. M.; Mylon, E., and Winternitz, M. C.: Yale J. Biol. & Med. 12:345, 1940	+

rule out a rheumatic process with absolute certainty. The leukocytic collections that always accompany the Aschoff bodies may in exaggerated cases appear in the form of a heavy diffuse infiltration (Sacks¹¹). Von Glahn¹² described masses of distorted cells and stated that he considered these, together with polymorphonuclear leukocytes, to be as distinctive as the Aschoff bodies. Since elongated and distorted cells were frequently found in the series reported here, the question arises whether there might be a connection between nonspecific myo-

11. Sacks, B.: Am. Heart J. 1:750, 1926.

12. von Glahn, W. C.: Am. J. Path. 2:1, 1926.

carditis and rheumatic myocarditis. It appears possible that both constitute a response of the interstitial tissue to an inflammatory focus elsewhere in the body.

In addition to the involvement of the heart there were in practically all cases lesions of other organs, particularly of the lungs. As bronchopneumonia is often a terminal event the importance of this lesion as a focus of inflammation may be subject to doubt. Since, however, bronchopneumonia alone was present in only 6 cases, this criticism could not materially subtract from the impression that myocarditis is generally connected with other inflammatory processes. Pulmonary lesions are mentioned in only few reports in the literature (Saphir¹³; Finland and others¹⁴; Covey¹⁵), and Saphir¹³ stated that inflammatory disease of the lungs is not commonly associated with myocarditis.

Much has been written about causation of nonspecific myocarditis, and the possible causes mentioned in the literature are briefly reviewed

TABLE 4.—*Results of Bacteriologic Studies Made During Life*

	Cases in Which No Bacteria Were Demonstrated	Cases in Which Given Bacterium Was Shown		
		Staphylococcus	Pneumococcus	Mycobacterium Tuberculosis
Smears from various sources (throat, skin, sputum)	1	3	3	3
Blood cultures	2	2	1	

in table 3. In addition, myocarditis arising in the course of pregnancy has been described (Gouley and associates¹⁶) and in uremia (Solomon and associates¹⁷). None of these possibilities have so far proved to be convincing. The main difficulty of an experimental approach lies in the fact that myocarditis occurs spontaneously with considerable frequency in experimental animals (Miller¹⁸). In the series under discussion the only constant finding was that of accompanying inflammatory lesions of other organs. The elevation of the leukocyte count observed in most cases points to the presence of an active inflammatory process (table 4).

The effect of sulfonamide compounds could be definitely ruled out in 12 cases. The fact that eosinophils were found in noteworthy numbers

13. Saphir, O.: *Arch. Int. Med.* **72**:775, 1943.

14. Finland, M.; Parker, F.; Barnes, M. W., and Joliffe, L. S.: *Am. J. M. Sc.* **209**:455, 1945.

15. Covey, G. W.: *Am. J. Clin. Path* **12**:160, 1942.

16. Gouley, A. B.; McMillan, T. M., and Bellet, S.: *Am. J. M. Sc.* **194**:185, 1937.

17. Solomon, C.; Roberts, J. E., and Lisa, J. R.: *Am. J. Path.* **18**:729, 1942.

18. Miller, C. P.: *J. Exper. Med.* **40**:543, 1924.

in only 3 cases further speaks against the presence of a sensitizing agent. There was no evidence that syphilis played a causal role.

The paucity of clinical evidence is illustrated by reports of sudden death in apparently healthy persons (Moritz and Zamcheck¹; Didion¹⁹; Saphir²). In the group of cases reported here the clinical manifestations of cardiac involvement were scanty, and in no instance was even a tentative diagnosis of myocarditis made.

With few exceptions (Schenken and Heibner²⁰) the reports in the literature do not include bacteriologic studies. In several of the cases in the series presented here various types of bacteriologic tests were performed during life, and the results are given in table 5. In addition, in 3 cases of diffuse myocarditis and in 3 of focal involvement sections were stained for bacteria, but no organisms could be demonstrated.

Some of the clinical records contained evidence of an infectious process that was present some time prior to the onset of the final illness. This fact, together with the difficulty of demonstrating any organisms

TABLE 5.—*Leukocyte Counts*

Leukocytes per Cubic Millimeter	Cases
5,000 to 10,000.....	3
10,000 to 20,000.....	13
20,000 to 30,000.....	5
30,000 to 40,000.....	2

in the heart, suggests that the bacteria exert their influence from a distant focus by means of their toxins rather than by direct invasion of the myocardium. This mechanism of bacterial action is consistent with the occurrence of myocarditis in scarlet fever and in diphtheria.

SUMMARY

Thirty-six cases of nonspecific myocarditis were found in 3,800 consecutive autopsies. The lesions appeared to be primarily interstitial in nature and varied considerably in their cellular composition. A constant finding was the presence of elongated cells with distorted nuclei, morphologically not identical with Aschoff cells or with Anitschkow's myocytes. Four cases in which only minimal foci were observed were included since it appeared likely that in them the changes might represent early stages of more extensive myocardial involvement. Extracardiac inflammatory lesions were found in all cases except 1. No other constant findings were present that could be regarded as possible causal factors.

19. Didion, H.: Virchows Arch. f. path. Anat. **310**:85, 1943.

20. Schenken, J. R., and Heibner, W. C.: Am. Heart J. **29**:754, 1945.

Case Reports

TUMORS OF THE THYMUS

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TUMORS of the thymus are of interest to clinicians and pathologists. To the former they have presented problems of diagnosis (as the case to be reported illustrates). To the latter they have presented certain difficulties with regard to their classification on an anatomic basis.

Thymic tumors may originate from the cells constituting the parenchyma, viz., the reticulum cells, the small thymic cells (the so-called thymic lymphocytes) or the corpuscles of Hassall. Primary tumors may also arise from the cells of the stroma and then may be in the nature of lymphosarcoma, fibrosarcoma, myxosarcoma or lipoma. Still another class of tumors has been described by Sternberg.¹ These apparently arise from the thymic parenchyma and are subsequently followed by a leukemic blood picture.

Wu² pointed out that the thymus is essentially an epithelial reticulum infiltrated by lymphocytes. He looked on the gland as a lymphoepithelial organ like the pharyngeal, faucial and laryngeal tonsils and the solitary lymph follicles of the pharynx, whose surface epithelium is normally infiltrated by lymphocytes. He described an innocent thymic tumor—diagnosed as lymphoepithelioma—with a characteristic morphologic aspect, composed of syncytial strands or cords of epithelial cells with varying numbers of lymphocytes. He also reviewed reports of 9 tumors diagnosed as lymphoepithelioma by previous writers, one tumor being cancerous, and he extracted from the literature reports of 10 thymic tumors given other designations in which the histologic description was compatible with the appearance of lymphoepithelioma. Of these 10 tumors, 2 were cancerous.

Margolis³ expressed the opinion that there is no justification for subdividing tumors of the parenchyma into carcinoma and lymphosarcoma in the absence of conclusive knowledge of the histogenesis of all of the constituents of the thymus. He therefore referred to thymic tumors by the generic term "thymoma"—a term originally introduced by Grandhomme.⁴

The difficulties experienced by various observers in their attempts to classify thymic tumors on an anatomic basis have thus resulted in a variety of names being used for the designation of these tumors.

From the Department of Pathology, University of Ceylon.

1. Sternberg, C.: *Wien. klin. Wchnschr.* **21**:475, 1908.

2. Wu, T. T.: *J. Path. & Bact.* **61**:351, 1935.

3. Margolis, H. M.: *Am. J. Cancer* **15**:2106, 1931.

4. Grandhomme, F., cited by Wu,² p. 363.

REPORT OF A CASE

A fairly well built man aged 45 years, a laborer, was admitted to the General Hospital, Colombo, on Aug. 14, 1945 with difficulty in breathing of three days' duration. The onset was sudden. He had suffered from hoarseness for the past eight months. There was no previous history of muscular weakness. There was marked dyspnea with inspiratory stridor and cyanosis. The veins in the neck were engorged. There were no swellings in the neck. Indirect laryngoscopy showed paralysis of the vocal cords. There were no other paralyses. Low tracheotomy was performed to relieve the dyspnea, which however continued. The patient was examined fluoroscopically on the third day. A large pulsating mass was seen occupying the anterior mediastinum (fig. 1). On account of the position of the shadow and the pulsation, a diagnosis of aortic aneurysm was made. The Wassermann and Kahn tests were negative. A white blood cell count showed 9,200 leukocytes per cubic millimeter with a differential count of 68 per cent polymorphonuclears, 22 per cent lymphocytes and 10 per cent eosinophils. The dyspnea increased, and the patient died on August 21.

Necropsy.—The body was that of a fairly well nourished person of about the age stated. There was pallor of the skin and the conjunctivas. The tips of the fingers showed cyanosis. There was a recent tracheotomy wound. The pericardial cavity contained about half an ounce (15 cc.) of blood-stained fluid. In the upper and anterior mediastinum was a tumor measuring $3\frac{1}{2}$ by $2\frac{1}{2}$ inches (9 by 6.5 cm.). It extended from the roots of the pulmonary artery and the aorta to a point about $\frac{1}{2}$ inch (1 cm.) below the tracheotomy wound (fig. 2). The tumor extended slightly over, and was adherent to, the pericardium. The ascending portion of the aorta passed through it. It was adherent to both lungs and was pressing on the right eparterial bronchus. There was a certain degree of bronchial dilatation in the upper lobe of the right lung with fibrosis (fig. 1). The tumor was encapsulated, but the penetration of the capsule by tumor tissue at several points gave rise to an irregular surface. The cut surface had a yellowish white appearance and in parts resembled fatty tissue. There were yellowish white deposits of tumor tissue over the left side of the trachea. It was noted that the main tumor mass extended in the form of a cord toward the thyroid gland. A careful examination with the naked eye, however, failed to reveal any deposits of tumor tissue in the substance of the thyroid gland.

Histologic Examination.—The type cell of the tumor resembled an epithelium or a reticulum cell (fig. 3), but the general pattern of the tumor varied in different parts on account of the variability of the cell arrangement, the presence of necrosis and degenerative changes in the cells and the arrangement of the fibrous tissue. Most of the cells were polygonal; some were ovoid or round. The nuclei were large and vesicular, with distinct nucleoli. The cytoplasm was pale pink. Multi-nucleated cells were frequent, and mitotic figures were seen in large numbers. In certain parts the tumor cells bore a close resemblance to squamous epithelium, but no intercellular bridges were seen.

The tumor cells were arranged in solid alveoli, the walls of which were formed of thick bands of fibrous tissue. In certain parts the cells occurred in small groups and strands, and in others large areas composed of sheets of proliferating cells were seen.

The arrangement of the tumor cells thus gave rise to different histologic patterns. Degenerative changes in the tumor cells, such as pyknosis, were evident in places. Necrosis was a marked feature. Large areas of necrosis infiltrated by polymorphonuclear leukocytes were seen in almost every section.

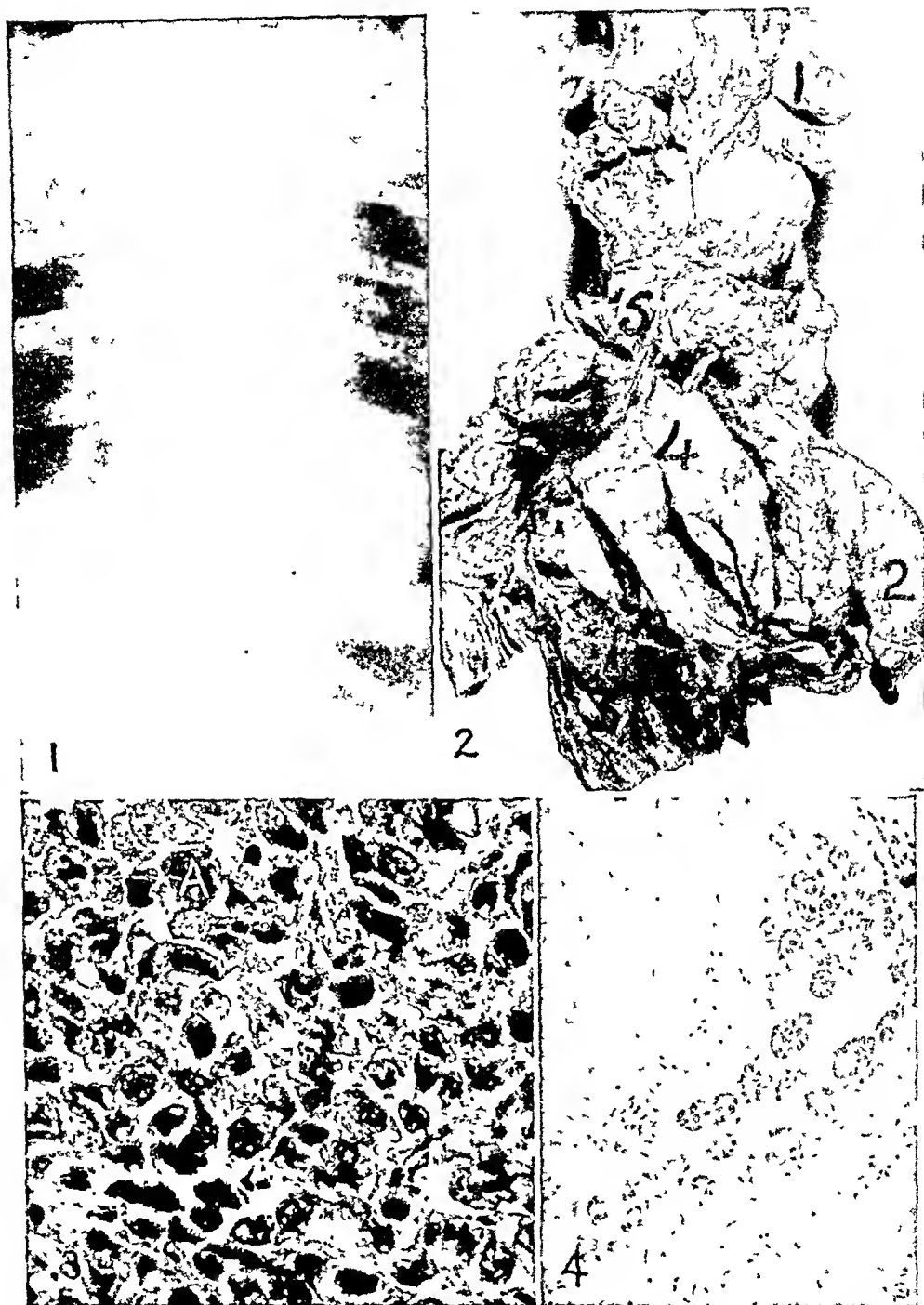


Fig. 1.—Roentgenogram showing a large mass in the anterior mediastinum. Note also the fibrosis at the right apex.

Fig. 2.—Appearance of the tumor at necropsy: (1) thyroid gland; (2) arch of the aorta; (3) pericardium; (4) tumor; (5) cordlike extension of the growth toward the thyroid gland.

Fig. 3.—Section of the tumor showing the cells of which it was composed. A mitotic figure is seen at A, top on the left. $\times 450$.

Fig. 4.—Tumor cells in a blood vessel. $\times 100$.

The stroma was composed of fibrofatty tissue, the fibrous tissue being in abundance. It was cellular in some parts and dense and hyaline in others. In certain parts the fibrous tissue formed the boundaries of alveoli containing tumor cells, and in others there was diffuse fibrosis, the fibrous tissue separating the tumor cells into small groups. Lymphocytes were few and were scattered here and there amidst the tumor cells. Only a small number of blood vessels were seen, and these, too, were rudimentary. (This probably explained the presence of large areas of necrosis and hemorrhage.) Although several sections from different parts of the tumor were examined, no structures resembling Hasall's corpuscles were seen. There was histologic evidence that the right lung and pleura, the pretracheal and tracheobronchial lymph nodes, the extrapericardial fat and the thyroid gland were infiltrated. Although the tumor encircled the first part of the aorta, it did not infiltrate the wall of the vessel. In the case of the lung and the pleura the tumor cell invasion was associated with much fibrosis. The lymph nodes were completely replaced by growth, the greater portion of which had undergone necrosis, leaving small groups of tumor cells here and there. The capsule and the periglandular tissue, too, showed infiltration.

Necrosis was a marked feature also of the tumor tissue infiltrating the fat. Here the cells were arranged in alveoli, strands or groups. Although involvement of the thyroid gland was not evident at autopsy, sections showed definite infiltration of the gland vesicles as well as of the pretracheal muscles.

In all these secondary deposits the tumor cells resembled those of the primary growth. There were innumerable mitotic figures and tumor emboli (fig. 4).

COMMENT

There is no doubt regarding the high degree of malignancy of the tumor. The large number of mitoses, the infiltration of several tissues and the presence of tumor emboli are capable of but one interpretation, that the tumor was highly cancerous.

There is no resemblance of this tumor to the lymphoepithelioma described by Wu.² Although the tumor cells resembled epithelium and grew in masses, the lymphocytes were few and may well have represented the lymphocytic elements of the thymic stroma.

As regards its histogenetic source, the difficulties encountered by Margolis³ do not appear to arise in this case. According to him, tumors whose cells are morphologically indistinguishable from lymphocytes may originate either from the stromal lymphocytes of the gland or from the small thymic cells in the gland parenchyma. In this case the tumor cells were morphologically quite different from lymphocytes or small thymic cells. The histologic features which have to be considered in determining the source of the tumor are (1) the striking resemblance of the tumor cells to the reticulum cells of the thymus or to epithelium, (2) their arrangement in distinct alveoli or solid nests, (3) the paucity of lymphocytes and (4) the marked fibrosis. These appearances indicate that the neoplasm was distinctly epithelial and had started in all probability from the thymic reticulum cells, which are derived from epithelium. According to Arey,⁵ the primordia of the thymus appear toward the end of the sixth week of intrauterine life as ventral saccula-

5. Arey, L. B.: *Developmental Anatomy*, Philadelphia, W. B. Saunders Company, 1938, pp. 190-192.

tions of the third pair of pharyngeal pouches. The sacculations are hollow at first but rapidly become solid epithelial strands. By the tenth week the original epithelium is transforming into a supportive framework of reticular tissue. The interpretation of the morphologic appearance of this tumor in the light of knowledge of the epithelial origin of the thymic reticulum cells leaves no doubt that its histogenetic source is the thymic reticulum.

Some noteworthy features which deserve comment are:

1. The infiltration of the thyroid gland. Although macroscopic examination failed to reveal secondary deposits, there was unmistakable microscopic evidence of infiltration of this gland. The extension of the growth in the form of a cord along the trachea toward the thyroid gland is significant. This growth may represent either involvement by the tumor of aberrant thymic nodules, which are specks of thymic tissue left behind by the gland during its descent into the thorax (Crotti⁶), or the distention by tumor cells of the "closed" system of lymphatic vessels between the thyroid and the thymus described by Williamson and Pearse,⁷ the existence of which, however, has been challenged by other workers (Crotti⁶).

2. The position of the tumor—surrounding the first part of the aorta—which gave rise to the skiagraphic appearance of an aortic aneurysm.

3. The absence of (a) symptoms of myasthenia gravis, (b) any abnormality of the sexual glands.

SUMMARY

A review of the literature reveals the difficulties experienced by various authors in the classification of thymic neoplasms.

A tumor of the thymus infiltrating several tissues, including the thyroid gland, is reported. On account of its anatomic relationship to the first part of the aorta, there was an appearance of pulsation on fluoroscopic examination which was mistaken for the pulsation of an aortic aneurysm. Reasons have been adduced to show that the tumor originated from the reticulum cells of the thymus.

6. Crotti, A.: *Diseases of the Thyroid, Parathyroid and Thymus*, Philadelphia, Lea & Febiger, 1938.

7. Williamson, G. S., and Pearse, H. W.: *Brit. J. Surg.* **17**:529, 1930.

PRIMARY CARCINOMA OF THE DUODENAL BULB

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SUPRAPAPILLARY carcinoma of the duodenum is a rare disease. Stewart and Lieber¹ found 1 case in 3,526 autopsies and 4 more cases in 20,176 autopsies. Our case, proved to be one of carcinoma of the duodenal bulb, represents 1 case in 4,786 autopsies.

REPORT OF A CASE

A white woman aged 64 was admitted to the service of Dr. L. D. McGuire at the Creighton Memorial St. Joseph Hospital, March 8, 1946, complaining of constant pain in the right groin which radiated to the leg. There was no abdominal pain, nausea or vomiting, and there was no history of tarry stools. The appetite was good, but the patient had lost 16 pounds (7 Kg.) of weight "recently." The patient had had one previous admission about a month before and was hospitalized at that time for eleven days for thrombophlebitis involving the right lower extremity. Examination revealed a well developed but undernourished elderly white woman. Her temperature was 98 F., pulse rate 68 and blood pressure 120 systolic and 82 diastolic. There was slight tenderness in the right upper quadrant of the abdomen, but there were no palpable masses. The solid organs were not palpable. On the lateral aspect of the right arm there was a single pea-sized subcutaneous nodule, which was firm and movable. The red blood cell count was 3,750,000; the hemoglobin content 78 per cent. The white blood cell count was 10,750, with eosinophils 2 per cent, monocytes 1 per cent, lymphocytes 28 per cent and neutrophils 69 per cent. Several urinalyses gave normal results except that one specimen showed a faint trace of albumin. On March 9, 1946 a biopsy of the nodule was made and reported as showing "grade III metastatic adenocarcinoma of the skin." On March 11, 1946 the patient complained of sharp pain in the epigastrium, which lasted only a short time. This was the first time the patient had experienced such pain. Eleven days later there was a similar episode of epigastric pain, but this time it radiated posteriorly to the back. The subsequent course of the patient was rapidly downhill, with a short period of coma prior to death.

Autopsy.—Gross Examination: The body was that of a well developed but undernourished elderly white woman. There was no jaundice. The primary lesion was found in the first portion of the duodenum. In the middle of the posterior wall of the bulb there was a rounded 1 cm. area with raised hard edges. The mucosa of the center of this area was ulcerated and red and measured about 3 mm. in diameter. The remainder of the mucosa was gray-white. The liver weighed 1,550 Gm. and was studded both on the outer and the cut surfaces with a large number of firm white nodules, which varied markedly in size, the largest measuring about 3 cm. in diameter. On the lateral side of the common duct there

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1. Stewart, H. L., and Lieber, M. M.: Arch. Surg. 35:99, 1937.

was a single ovoid lymph node, which measured 1 by 2.5 cm. The node was firm and on cut surface a uniform pale white. The common duct was not dilated. Tumor tissue was not definitely identified grossly in any of the other organs, although microscopically tumor was found in both lungs, both kidneys and the medulla of one adrenal gland.

Microscopic Examination: Sections through the lesion of the bulb showed in the mucosa many scattered small acini lined by cancerous cells. These cells were generally large and hyperchromatic and differed considerably in shape. The nuclei varied from vesicular to pyknotic, and they were all large. Some mitotic figures were seen. There was ulceration of the mucosa with a diffuse sprinkling of polymorphonuclears throughout this layer. The submucosa showed extensive destruction of Brunner's glands and replacement with tumor, which was made up of poorly formed acini. These acini were of various sizes and shapes and generally larger than those seen in the mucosa. Small, irregular islets of tumor cells were



Duodenal adenocarcinoma.

also seen. The individual cells, however, were similar to those seen in the mucosa. The stroma was of a fine fibrous variety and interrupted by amorphous acidophilic debris and red corpuscles (fig. 1). There was no invasion of the muscularis in any of the sections studied. In other organs in which tumor was found, the histologic picture was essentially the same as in the original tumor.

The final diagnosis was adenocarcinoma of the first portion of the duodenum with metastases in a regional lymph node, the liver, both lungs, both kidneys, the medulla of one adrenal gland and the subcutaneous tissue of the right arm.

COMMENT

Stewart and Lieber¹ collected 35 acceptable cases in the literature of primary suprapapillary carcinoma of the duodenum and added 6 original cases. Berger and Koppelman² added 14 more acceptable cases. Since 1942 we have found 8 cases, and to this we are adding our case.

2. Berger, L., and Koppelman, H.: *Ann. Surg.* **116**:738, 1942.

Eight Cases of Carcinoma of the Duodenum

Author	Age	Sex	Symptoms	Röntgenographic Findings	Pal- pable Blood Mass in Stool	Comment
1. Hartzell, H. V.: Radiology 39: 474, 1942	69	F	Anorexia, weakness and nausea	Palpable mass corresponding to duodenal bulb, which showed constant filling defect, irregular mucosal pattern and 6 hr. retention	+	Autopsy: Pyloric ring formed base of mass extending 6 cm. down duodenum; mass nodular and almost occlusive; extension to liver. Diagnosis: Adenocarcinoma
2. Burke, E.; Perkel, L. L. and Gnassi, A. M.: Am. J. Surg. 62: 267, 1943	27	M	Epigastric pain after eating, relieved by soda; "hemorrhage from duodenal ulcer." Several admissions, one for perforation	Deformity with ulcer crater	0	Operation: Gallbladder adherent to anterior wall of duodenum, under which was perforated ulcer with gallbladder as floor. Opposite posterior wall another ulcer had burrowed into pancreas. Patient well in 1943
3. Ritvo, M., and Hewes, F. L.: Radiology 38: 7, 1942	64	M	Epigastric pain relieved by milk, soda and crackers; loss of weight	Extensive irregularity of cup, "ulcerative in character"	0	Autopsy: 4 cm. ulcerative lesion 1st portion of duodenum: adenocarcinoma
4. Cohn, I.: Ann. Surg. 119: 342, 1944	71	F	Pain in right upper quadrant; intermittent vomiting unaccompanied by nausea; loss of 20 lb. (9 Kg.)	Dilatation of first portion of duodenum with altered mucosal pattern and retention of barium sulfate	+	Operation: 5 x 4 cm. pedunculated mass just distal to pyloric ring; enlarged nodes in greater omentum along greater curvature; microscopically, "involvement of the nodes"
5. Cohn, I.: Ann. Surg. 119: 342, 1944	62	M	Abdominal pain	Sharply demarcated filling defect in descending duodenum proximal to ampulla	0	Operation: 2 in. (5 cm.) beyond pylorus medially, an infiltrating mass, 1½ in. (4 cm.)
6. Lally, T. C.: Ohio State M. J. 39: 50, 1943	73	F	Epigastric pain, nausea and vomiting; loss 25 lb. (11 Kg.) in 6 mo.; icterus	Gastric retention; deformity and spasm of bulb, with niche of lesser curvature of bulb	+	Autopsy: Adenocarcinoma of suprapyloric portion of duodenum: extension to surrounding fibroadipose tissue, common duct, stomach, pancreas; metastases in liver and hepatic nodes; obstruction of biliary tree, cholangitis, multiple abscesses of liver, bile nephrosis; squamous carcinoma of cervix
7. Bajer, I.: M. Press 214: 46, 1945	25	M	Epigastric discomfort not affected by food; fatigue; anorexia; loss of weight, and later epigastric pain radiating to back	Residue in third portion of the duodenum	0	Operation: 3 cm. bleeding papillomatous growth partially obstructing lumen in 1st portion of duodenum: "cellular glandular carcinoma"
8. Thorstad, M. J.; Gardner, L. W. and Revendo, W. S.: Harper Hosp. Bull. 2: 12, 1944	67	F	At onset, several bouts of painless jaundice; loss of appetite, weakness and progressive loss of weight	Small diverticulum of third portion of duodenum only finding mentioned in connection with this portion of gastrointestinal tract	0	Autopsy: Round ulcerating 4 x 4 x 3 cm. tumor encroaching on ampulla: adenocarcinoma with colloid changes (not tumor of Brunner's glands)

making a total of approximately 64 cases of primary suprapapillary carcinoma of the duodenum reported in the literature.

Analysis of the last 8 cases reveals that: 1. The symptom complex varies from "abdominal pain" to symptoms of peptic ulcer. It is interesting to note that in cases 2 and 3 (table), in which the symptom complex simulated that of a peptic ulcer, the lesion found was essentially an ulcer. In the remainder of the cases the lesion was papillary, and the symptoms were indefinite. 2. In most of the cases there were roentgenographic findings of deformity of the duodenum, filling defect or ulcer crater, or a combination of all three. 3. Other findings, such as a palpable epigastric mass and a test showing occult blood in the stool, varied.

In our case symptoms somewhat suggestive of peptic ulcer were late. The appearance of a subcutaneous nodule which proved to be metastatic adenocarcinoma was the first indication of the presence of a cancer. The widespread metastases revealed at autopsy would indicate that the primary tumor had been present for some time, and yet clinically there were only late symptoms referable to the gastrointestinal tract.

Microscopically, tumor was seen in the mucosa and submucosa. Only an abrupt-transition was noted between normal glandular structure and tumor. There was no mucus or pigment. The appearance of the individual tumor cells resembled somewhat both the cells of Brunner's glands and the columnar epithelium of the duodenal mucosa. One is therefore unable to state definitely that this tumor arose from any particular type of epithelium of the duodenum.

SUMMARY

A case of primary carcinoma of the first portion of the duodenum is presented.

In 2 of the 8 cases collected from the recent literature, there were symptoms of peptic ulcer. The lesion found in these 2 cases was essentially an ulcerative lesion. In the remainder of the cases the symptom complex was indefinite, and the lesion found was a papillary growth.

In our case there were no symptoms referable to the gastrointestinal tract until late. A single metastatic nodule in the subcutaneous tissue of an arm was the only clue to the presence of a carcinoma.

No decision could be reached as to the type of epithelial cell of the duodenum from which the tumor originated.

426 South Camden Drive.

Laboratory Methods and Technical Notes

A SIMPLIFICATION OF THE TECHNIC FOR DEMONSTRATING ALKALINE AND ACID PHOSPHATASE IN TISSUES

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THE HISTOCHEMICAL methods demonstrating alkaline phosphatase, first described by Gomori¹ and by Takamatsu,² and acid phosphatase (Gomori³) have become, with certain minor modifications, routine to the experimental pathologist and cytologist. The existing technics, while relatively simple, require that the buffered staining solutions be prepared from a series of previously prepared stock solutions. Those for the alkaline phosphatase method are quite different from those for the acid phosphatase.

The method described in this paper is a simplification of the existing technics in that the stock buffer solution for each enzyme is made up with the metallic ion which acts as the indicator, the activator of the enzyme and the buffer all in one solution. The substrate is so prepared that it may be used with either the buffered stock solution for alkaline or that for acid phosphatase.

TECHNIC

I. SOLUTIONS

A. Alkaline phosphatase buffer stock solution

Barbital sodium.....	5.0 Gm.
Calcium chloride.....	5.0 Gm.
Magnesium chloride.....	0.5 Gm.
Water, distilled.....	1,000.0 cc.

It is advisable to check the p_H and adjust to p_H 9.4 if necessary.

B. Acid phosphatase buffer stock solution

Sodium acetate.....	27.2 Gm.
Acetic acid, glacial.....	12.0 cc.
Lead nitrate.....	5.0 Gm.
Water, distilled.....	1,000.0 cc.

It is advisable to check the p_H and adjust to 4.5 to 5.0 if necessary.

C. Stock glycerophosphate substrate solution

Sodium glycerophosphate (52 per cent alpha).....	20.0 Gm.
Water, distilled.....	1,000.0 cc.

About 5 drops of chloroform are added to each of the foregoing solutions as a preservative, and the solutions are stored in the refrigerator when not in use.

1. Gomori, G.: Proc. Soc. Exper. Biol. & Med. **42**:23, 1939.
2. Takamatsu, H.: Tr. Soc. path. jap. **29**:492, 1939.
3. Gomori, G.: Arch. Path. **32**:189, 1941.

For use, the following buffer-substrate mixtures are prepared from the foregoing stock solutions.

D. Alkaline phosphatase buffer-substrate mixture

Alkaline phosphatase buffer stock solution (solution A).... 4 parts

Stock glycerophosphate substrate solution (solution C).... 1 part

E. Acid phosphatase buffer-substrate mixture

Acid phosphatase buffer stock solution (solution B).....10 parts

Stock glycerophosphate substrate solution (solution C).... 3 parts

II. STAINING

A. Alkaline phosphatase

1. Fix sections of tissue cut 2 to 3 mm. thick in cold acetone (4 C. or less) for twenty-four hours and then impregnate at room temperature with 5 per cent solution of cellulose acetate in acetone for six hours.

2. Drain the excess cellulose acetate from the pieces of tissue and transfer tissue to xylene for one hour.

3. Embed in paraffin at 54 to 56 C. for sixty minutes and prepare paraffin blocks in the usual manner.

4. Cut sections at 5 to 8 microns and mount on slides with albumin-glycerin. The tissue is fixed to the slide by placing in an incubator at 37 C. for five to eighteen hours.

5. Remove paraffin from sections by the usual method with xylene, absolute alcohol or acetone and 95 per cent alcohol.

6. Incubate the sections in the alkaline phosphatase buffer-substrate mixture (solution D) at 37 C. for one to twenty-four hours, depending on the concentration of enzyme in the tissue.

. A duplicate set of sections to serve as controls is incubated under the same conditions for the same length of time in the alkaline phosphatase buffer stock solution with distilled water substituted for the stock glycerophosphate substrate solution.

7. Rinse rapidly in distilled water.

8. Immerse sections in 5 per cent aqueous solution of silver nitrate and expose to light. Sunlight may be used. This requires up to one hour's exposure. The use of a General Electric "mazda RS sunlamp," 275 watts, as a source of ultraviolet radiation is ideal. With the latter method, two minutes' exposure is recommended.

9. Fix reduced silver by placing the sections in a 5 per cent aqueous solution of sodium thiosulfate for one minute and wash under running tap water for five minutes or in about ten changes of water.

10. Counterstain lightly, if desired, with either Harris' hematoxylin or 1 per cent aqueous light green SF. If hematoxylin is used, wash in water after staining, and if light green is used, differentiate quickly in alcohol.

11. Dehydrate in 95 per cent alcohol, followed by absolute alcohol or acetone.

12. Clear in xylene and mount in balsam or "clarite."

Results.—The sites of alkaline phosphatase activity stain golden or dark brown to black.

B. Acid phosphatase

Steps 1 through 5 are identical with those for alkaline phosphatase described previously.

6. Incubate the sections in the acid phosphatase buffer-substrate mixture (solution E) at 37 C. for six to twenty-four hours, depending on the enzyme concentration in the tissue.

A duplicate set of sections to serve as controls is incubated under the same conditions for the same length of time in acid phosphatase buffer stock solution with distilled water substituted for the stock glycerophosphate substrate solution.

7. Rinse in distilled water.

8. Rinse in 2 per cent acetic acid.

9. Wash thoroughly in distilled water.

10. Immerse for two minutes in a dilute solution of ammonium sulfide and wash under tap for five minutes.

11. Counterstain as, and if, desired (see step 10 under method for alkaline phosphatase), dehydrate and mount in balsam or "clarite."

Results.—The sites of acid phosphatase activity stain dark brown to black.

COMMENT

The histochemical methods described are used routinely in the laboratory of the section on pathologic anatomy of the Mayo Clinic to demonstrate alkaline or acid phosphatase activity or both. More than 1,000 sections have been successfully stained by these procedures. With human tissues, good results have been obtained even as long as twenty-four hours post mortem and even though the tissues were taken from bodies in which there had been arterial embalming prior to necropsy.

It has been found that the combining of the buffers with the calcium and lead salts, for alkaline and acid phosphatase, respectively, reduces the number of reagents needed in the immediate preparation for the staining, simplifies the procedure, reduces the possibility of error and gives uniformly good results. The glycerophosphate solution is reasonably stable when stored in the refrigerator, and the buffer-substrate mixtures are always clear.

In the final steps of the demonstration of alkaline phosphatase, we at the Mayo Clinic employ in essence the von Kossa⁴ method for calcium. As a source of light, we use a General Electric "mazda RS sun-lamp" (275 watts) instead of daylight, a procedure which principally produces uniformity of exposure and is of time-saving value, simultaneously. The lamp is mounted in a wooden box (figure) constructed for this purpose. The staining dish containing the slide is introduced beneath the light on the tray at the bottom of the box. The box is of simple construction, and the materials used are easily obtainable. There are no rigid specifications; it can be designed to meet the needs of the individual laboratory. The one we use measures 15 by 9½ by 9 inches (38 by 24 by 23 cm.), in height, depth and width, respectively.

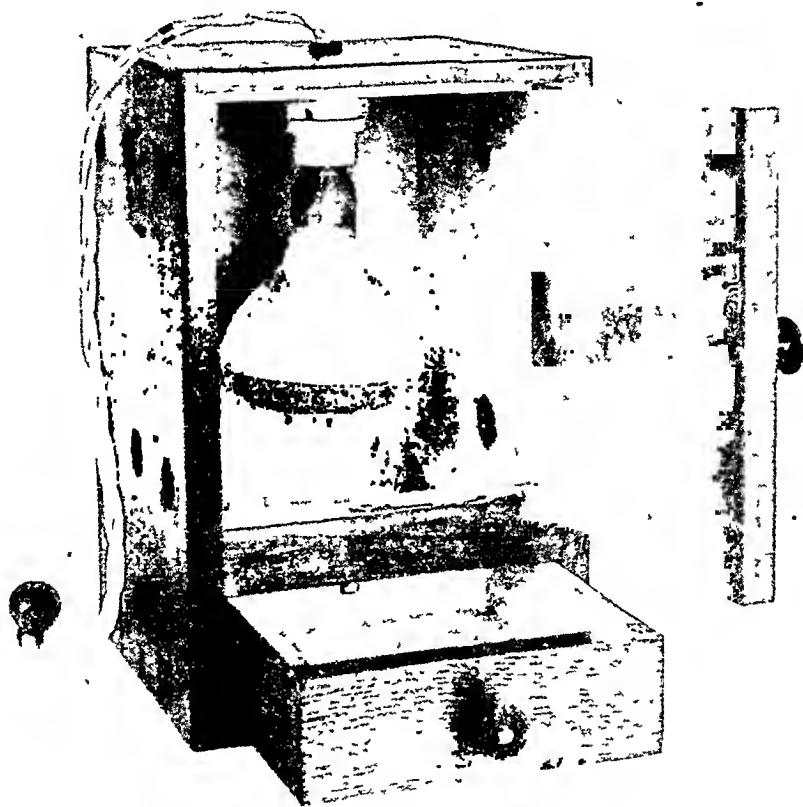
The sites of alkaline phosphatase activity are readily demonstrated. The results of the procedure for demonstrating acid phosphatase are less uniform and at times disappointing unless meticulous attention to detail is observed. In working with this enzyme, one should empha-

4. von Kossa, J.: Beitr. z. path. Anat. u. z. allg. Path. **29**:163, 1901

size particularly fixing of the tissues at low temperatures and not departing from the staining technic given. Moog⁵ has shown that ascorbic acid in one hundredth-molar concentration will activate the acid phosphatase, and this may be added to the acid phosphatase buffer stock solution, particularly if it is anticipated that the concentration of the enzyme will be low.

SUMMARY

A simplification of existing technics for demonstrating alkaline and acid phosphatase is presented. The essential features of the modifi-



Front view of staining box described in the text for the von Kossa stain. The drawer for the staining dish is partially open; a door (open in the photograph) is convenient for changing the "sunlamp," when and if necessary, and for cleaning the box.

cation are such that the number of stock solutions is reduced. The solutions used in this method are stable and may be made up in large quantities; thus the necessity of preparing staining solutions from a relatively large number of stock solutions each time the test is performed is avoided. The stock glycerophosphate substrate solution is used in both the method for alkaline and that for acid phosphatase.

5. Moog, F.: *Biol. Bull.* 86:51, 1944.

PROCEDURE FOR DEMONSTRATING LEPROSY BACILLI IN PARAFFIN SECTIONS

G. L. FITE, M.D., P. J. CAMBRE and M. H. TURNER, B.S., CARVILLE, LA.

THE lesser degree of acid-fastness of leprosy bacilli as compared with tubercle bacilli appears in a disagreeable manner in the difficulty of demonstrating the organisms of leprosy in paraffinized tissues.¹ Faraco² showed that by ordinary methods of demonstrating acid-fast organisms the leprosy bacilli are often not acid fast, are not differentiated and may be stained by the counterstain. He devised a method of oiling the sections and staining with carbolfuchsin while the sections contained oil. Under these conditions the bacilli retained this dye. The method is effective but awkward and cumbersome. In working with similar procedures it has been found that staining before removal of the paraffin is satisfactory (though impracticable) and that replacement of the paraffin with a light oil produces still better results. The following procedure, which is not exactly a new method, has proved most valuable in demonstrating leprosy bacilli in tissues, irrespective of the technics of fixation and embedding. It succeeds admirably with tissues indifferently fixed or embedded years previously where other procedures fail miserably.

PROCEDURE

1. Remove paraffin with two changes of the following mixture, allowing a minute or two for each change:

Cottonseed (or peanut or olive) oil..... 1 part

Xylene 2 parts

2. Drain, wipe off excess oil and blot to opacity. The residual oil in the section helps to prevent shrinkage and injury of the section.

3. Remove mercury crystals (if present) with strong solution of iodine, U. S. P. (two minutes), followed by a hyposulfite or thiosulphate solution rather than alcohol. Wash in tap water.

4. Stain cold fifteen to thirty minutes in any standard preparation of carbolfuchsin (Ziehl-Neelsen) but not in a concentrated solution such as Kinyoun's. Wash.

5. Decolorize with 1 per cent concentrated hydrochloric acid added to 70 per cent alcohol—not to the point of totality, but leaving a faint pink color. One to two minutes will be required. Wash.

6. Counterstain with Loeffler's alkaline methylene blue about thirty seconds. Wash in tap water.

From the Pathology Laboratory, United States Marine Hospital (The National Leprosarium), United States Public Health Service.

1. Fite, G. L.: *Am. J. Path.* **14**:491, 1938.

2. Faraco, J.: *Rev. brasil. leprol.* **6**:177, 1938.

7. Blot, let stand a few minutes to dry out well, mount directly in a synthetic mounting medium, such as "clarite" or "permount."

Almost any oil will serve the purpose, from liquid petrolatum to camphorated oil, although the volatile oils are less useful. The oil slows up the various steps a little, but not importantly. It hastens the acid-fast staining, however, so that the use of heat is preferably avoided, more even regular staining resulting without it. A larger proportion of oil in the xylene makes even decolorization difficult. It is possible to take sections to water by routine methods and then oil them, as Faraco did, but it is difficult to obtain even staining thereafter. The procedure as given is equally effective with the bacilli of rat leprosy and should do well with tubercle bacilli. It is particularly recommended for tissues containing the organisms of human leprosy.

Notes and News

Appointments, Etc.—Stanhope Bayne-Jones has resigned as professor of bacteriology in Yale University and as director of the board of scientific advisers of the Jane Coffin Childs Memorial Fund for Medical Research to accept the position of president of the joint administrative board of the New York Hospital-Cornell University Medical Center. His address now is 525 East Sixty-Eighth Street, New York.

Laurence H. Snyder, professor of zoology and of medical genetics at Ohio State University, has accepted the deanship of the graduate school of the University of Oklahoma and the professorship of genetics of the college of medicine.

In the United States Public Health Service Leonard Scheele has been appointed chief of the National Cancer Institute in succession to R. R. Spencer, who resigned to devote himself to biologic research and to the training of young physicians in the enlarged cancer program of the institute.

Central Laboratory.—The Veterans Administration has established a central pathologic laboratory in Washington, D. C., in cooperation with the Army Institute of Pathology, to provide more facilities for consultation, training and research in pathology.

Research Fellowships.—The National Foundation for Infantile Paralysis, Inc., has made a grant in support of research fellowships in fields related to infantile paralysis. Fellowships are offered to provide (1) opportunities for training and research in those basic medical sciences which will be of particular value in furthering progress in the field of orthopedic surgery and (2) special experience in the study of virus diseases. Senior fellowships in the fields of orthopedic surgery, pediatrics and virus diseases, open to men and women who have already shown definite achievement in research, are also offered under this grant. The chairman of the board in charge of these fellowships is Robert F. Loeb, Columbia University, New York.

The American Institute of Biological Sciences.—This institute is to be set up within the framework of the Division of Biology and Agriculture of the National Research Council, which is financing the preliminary organization. H. B. Steinbach, Washington University, St. Louis, with headquarters at the National Research Council, is acting as executive secretary during the period of organization.

New Journal.—A new periodical on pathology and clinical medicine is published by the department of pathology, Hospital 2 de Mayo, Lima, Peru, under the title *Archivos peruanos de patologia y clinica*. The editor is Dr. Oscar Urteaga-Ballon.

The American Society for the Study of Arteriosclerosis.—This society has been organized with a membership of about 80 and with W. C. Hueper, 113 West Eighteenth Street, New York, as president, William B. Kountz, St. Louis, as vice president, and O. J. Pollak, Wilmington, Del., as secretary-treasurer. The directors are: E. Cowles Andrus, Baltimore; G. Lyman Duff, Montreal, Canada; Harry Goldblatt, Los Angeles; George R. Herrmann, Galveston, Texas; Louis N. Katz, Chicago; Irvine H. Page, Cleveland. It is planned to hold a meeting in Chicago this fall.

Books Received

HIPPOCRATIC WISDOM: FOR HIM WHO WISHES TO PURSUE PROPERLY THE SCIENCE OF MEDICINE. A MODERN APPRECIATION OF ANCIENT SCIENTIFIC ACHIEVEMENT. By William F. Petersen, M.D. Pp. 263, illustrated. Price \$5. Springfield, Ill.: Charles C Thomas, Publisher, 1946.

"Hippocratic Wisdom" is a modern appreciation of ancient scientific achievement. The book voices and advocates very winningly, and yet emphatically, the indispensable tradition and continuity of classical medical theory and practice. It analyzes the mature wisdom connected with the name of Hippocrates and shows that the approach to scientific medical knowledge was the same in classical days as it ideally is now.

Dr. Petersen is aware that all great medical achievement has grown out of a recognition of the laws of nature and of nature itself. The Greeks did not know it all, but they were naturalists in their knowledge and experience, as they turned their skill to medical problems and the art of healing. Not technicians in our modern sense, they conceived man and his functions and ailments, and the regulation or cure of these, as deeply influenced by forces and conditions in nature. That was their basic clinical view, and out of that view their observations were recorded thoughtfully, their interpretations determined and formulated.

The hippocratic texts need no more elaborate commentary than their repeated study has deposited in literature thus far. Therefore Dr. Petersen proposes in his exposition of the Wisdom no new commentarial apparatus, but emphasizes the validity of the Greek tradition as a principle of principles for those who aim to serve mankind through medicine, not merely to practice a craft of skill.

Aptly the author compares modern medical teaching to the teaching of anthropology without ecology—or, one might say, botany without plant relationship or physiology. Skill is a great virtue, as is a perfect technic, but it must not be isolated from clinical consideration and experience. Not laboratory experience alone, nor study in the library, but persistent, intelligent observation in the sick room, backed by similar experience with normal manifestations and phenomena. "In the beginning are the cases," as Henderson says; at the end should be the descriptive exposition of the case, a theory based on a wider range of uniformity, and the conceptual scheme of aiding nature's healing powers. Hippocrates knew that air is a basic requirement of tissue function and that a disturbance of this factor influences pathologic conditions and may call forth many other factors (weather, etc.) disturbing the balance of the body humors; he also had a view of compensatory reactions between the body tissues. His theory of inflammation recognizes many kinds of trauma. More interesting even than these enlightening conclusions is that with the old master came a beginning recognition of the complexity of causes of disease which deserves special attention in view of modern theories of specific causes and specific cures, but these later theories frequently prove too simple as applied to complicated processes and conditions.

So Dr. Petersen leads us through the details of the Wisdom by a spirited analysis, amplified by modern experience, of what Hippocrates specifically said and meant.

It is advisable to follow this analysis with some deliberation, for the hippocratic texts alone contain plenty of food for serious reflection and comparison, and nobody need think the author is giving us some easily digested medical-historical entertainment. The texts read easily enough in translation but appeal directly to philosophic thought, which would better not be suppressed if the intended enlightenment is to result. Nor are the author's many incisive and elucidating remarks, which bring out both contradistinctions and similitudes between medical and

philosophic thought and practice in the fifth (B. C.) versus the twentieth century, to be neglected by the reader, whether student or master of the science of medicine.

The specific analysis of the texts includes twelve chapters comprising the ideas of Hippocrates on erysipelas (as related to the seasons), anoxia, reproduction, epilepsy, hydrophobia, pneumonia and allied diseases, phthisis, the case of Silenus and other observations; surgical technic, bandaging; regional surgery, fractures and dislocations; medical theory, the principle of disease, general trauma. These chapters are followed by a resumption or corollary that brings out what may be called in old fashioned language a moral—apart from the light it throws on the hippocratic group of medical writings. We quote for once the following:

"It is possible that we, who have lived during the epoch of great therapeutic advance as well as the students who enter the field of medicine when this development is proceeding at an accelerated pace, may feel that Hippocratic wisdom is wholly out of date.

"These very advances in therapeutic intervention have led away from a broad concept of the causation of disease. Who need care about the why of disease when the remedy is immediately at hand?

"The student sees a coryza in the department of otolaryngology; a spontaneous abortion in obstetrics; an endocarditis in medicine; a retinal thrombosis in ophthalmology; a prostatic episode in the genitourinary department; a hypomania in psychiatry; a diverticulitis in surgery, or a Bell's palsy in neurology without ever being faintly conscious of the connecting link that might make an intelligible and coherent picture of the tangled skeins. He never considers the patient as a whole in the environment as a whole."

In these analyses there are interpretations which might be considered critically, and other formal details probably debatable. But on the whole, the Hippocratic *direction* does not depend on syllables and other philologic niceties; it consists in movement rather than in form.

The last hundred pages contain notes and references, a vocabulary and a well prepared table of contents.

In a way this is an astonishing book, coming, as it does, from a medical man deeply concerned in the realities of his day and yet looking back twenty-three hundred years for historic anchors by which to keep secure the ship which he shares with many other capable men. But the Greeks never fail to inspire mankind historically. As Dr. Petersen says: "Our age is one very much like the Hippocratic.—Witness the clashing impact of empires and the march of the legions—the transition state of social forms—the ambiguous shibboleths, the confusion of the patriot, and the cunning of the tyrants."

This is true, although medical men never have gone to the radical extremities practiced by politicians. Our forces of social order cannot ever be stifled. Nor should the author of this constructively provocative book be denied the opportunity he seeks through this work of inspiring his colleagues, young and old, with the dignity, the benefit, the necessity, of giving proper respects to the ideals which the muse of history preserved for our welfare.

CHARLES-ÉDOUARD BROWN-SÉQUARD: A NINETEENTH CENTURY NEUROLOGIST AND ENDOCRINOLOGIST. By J. M. D. Olmsted, M.A. (Oxon.), Ph.D., D.Sc., professor of physiology, University of California. Pp. 253. Price \$3. Baltimore: The Johns Hopkins Press, 1946.

This volume contains the eighth course of lectures under the Hideyo Noguchi Lectureship of the Institute of the History of Medicine of the Johns Hopkins University. The lectureship was endowed by the late Emanuel Libman, of New York. There are three lectures, the headings of which give a good idea not only of their scopes but also of the main epochs in the life of Brown-Séquard: Mauritian student and free lance investigator in Paris (1817-1894); his neurologic practice and American professorships (1854-1878); his occupancy of the chair of medicine at the Collège de France (1878-1894). Brown-Séquard was born in Mauritius,

then under the British flag. His father, Charles Edward Brown, captain in the American Merchant Marine, was lost at sea before the son was born, and the French mother, Charlotte Séquard, was thrown on her own resources. The further story is crowded with exciting events: the study of medicine in Paris; remarkable physiologic experiments; lectures in Philadelphia, the father's birthplace, New York and Boston; brief service as professor in the Medical College of Virginia; delegate to the 1855 meeting of the American Medical Association. This visit to the United States was the first of many up to 1878 when "he had finally at the age of sixty-one come home to France" for good as the successor of Claude Bernard at the Collège de France. In concluding his scholarly biography and his circumstantial and critical analysis of Brown-Séquard's investigative work, Olmsted places him as "the last in the line of a great tradition of French experimental physiology at the Collège de France. . . . Magendie, Bernard and Brown-Séquard spanned the XIX century from its first decade to the last, and from their work came the ideas whose fulfillment now occupies much of our present day investigation." Brown-Séquard "left an indelible mark on physiology and medicine, and we look back on him as a brilliant and indefatigable investigator. . . ." Olmsted's book is welcomed as an important and timely contribution to medical biography and history.

HENRICI'S MOLDS, YEASTS AND ACTINOMYCETES: A HANDBOOK FOR STUDENTS OF BACTERIOLOGY. By Charles E. Skinner, Ph.D., assistant professor of bacteriology, University of Minnesota, Minneapolis; Chester W. Emmons, Ph.D., principal mycologist, Division of Infectious Diseases, National Institute of Health, Bethesda, Md., and Henry M. Tsuchiya, Ph.D., research associate, Division of Microbiology, Hormel Institute, University of Minnesota, Austin. Second edition. Price \$5. Pp. 409, with 136 illustrations. New York: John Wiley & Sons, Inc., 1947.

Henrici's book was published first in 1930. It was received with favor. The revision begun by Henrici has been completed in thorough and competent fashion. A new chapter deals with penicillin and other antibiotic substances, and another with variations in the lower fungi. The book is well printed and illustrated. It represents fully the present state of scientific, medical and industrial mycology, and will continue to be a standard work in its field.

THE PRESERVATION OF PROTEINS BY DRYING WITH SPECIAL REFERENCE TO THE PRODUCTION OF DRIED HUMAN SERUM AND PLASMA FOR TRANSFUSION. Medical Research Council Special Report Series no. 258. By R. I. N. Greaves. Paper. Price 2 shillings. Pp. 54, with 5 figures and 20 plates. London: His Majesty's Stationery Office (New York: Library of British Information, 30 Rockefeller Plaza), 1946.

Because of experience gained in freeze-drying antisera on a laboratory scale in the department of pathology of Cambridge University, the Medical Research Council commissioned the author in 1939 to develop efficient methods for freeze-drying the large amounts of plasma and serum likely to be needed during the war. An original pilot plant and then a final pilot plant which embodied the principles worked out by Greaves and his colleagues were produced. Following this a plant was constructed at Cambridge designed for an output of 2,500 bottles of dried material per week but which could be extended to 5,000 bottles per week. In this plant was produced most of the dried serum and plasma used by the British military forces and civilians, consisting of, up to September 1945, 318,700 bottles, each representing 400 cc. of material. An additional 32,617 bottles were produced in the pilot plants. This does not include dried diagnostic and therapeutic sera or sera and plasmas for experimental purposes.

In this monograph are set forth the theoretic considerations involved in desiccation from the frozen state and the physical principles of the processes. It also presents the design and technical details of the large drying plant. The problems of drying from the frozen state were solved by methods which in some respects differ from those used in the United States

The primary drying in the large plant was carried out in eight large steel cylindric desiccators measuring 3 feet (91 cm.) in diameter and 6 feet (183 cm.) high. Steel plates welded to the bottom and sealed to the top with "Apiezon Q" made the chambers air tight. Suspended from the top cover is a heater head constructed to carry 180 standard M.R.C. bottles, each one in a cell surrounded by an electric coil that supplies 7 watts of current regulated to heat the bottles to $+30^{\circ}\text{C}$. In the bottom of the chamber is a condenser consisting of a coil of copper tubing through which is pumped brine at -40°C . from a central ammonia refrigeration plant. Vacuum requirements are provided by two large single stage pumps connected by a manifold to the eight desiccators.

A novel way of preliminary freezing of the material to be dried is used. The bottles are rotated at a comparatively high speed on their vertical axes in giant centrifuges. In this way a cone is forced down through the liquid, which is frozen in this position by a current of air at -18°C . The same effect is obtained as in shell freezing with liquid refrigerants. When transferred to the drying chambers and the pressure reduced rapidly enough, the liquid remains frozen until dried.

In order to produce 5,000 bottles of dried material a week the whole drying cycle, including defrosting of the condenser, is completed and restarted in forty-eight hours. At half this rate, seventy-two hours could be allowed for the drying and twenty-four hours for defrosting and servicing.

When removed from the primary desiccators, the dried protein contains 0.4 per cent residual moisture. To remove this, the bottles are transferred to smaller chambers, where they are kept four days *in vacuo* over phosphorus pentoxide. At the end of this period the vacuum is replaced by dry oxygen-free nitrogen to a slight positive pressure.

The physical factors involved in drying are automatically controlled and mechanically recorded, including the temperature developed in the bottles as measured by thermocouples, the temperature of the circulating refrigerant as measured by a platinum resistance thermometer, and the pressures produced in the chambers as measured by Pirani gages. An alarm system gives warning of a failure of electric supply, a rise of temperature of the brine or a rise of pressure in the vacuum system.

The container for the material being dried is kept bacteriologically closed throughout the whole process. No antiseptic is added. During drying in the primary chambers the mouth of the bottle is covered with a pad of cotton between two layers of gauze. The pad is replaced with a rubber diaphragm held in place with an aluminum cap. For the final drying and introduction of nitrogen a hole is punched in the metal cap and a hypodermic needle to which is attached a bacterial filter is pushed through the rubber diaphragm. The final seal is made with plasticine and by dipping the neck of the bottle in a capping solution.

The material in the monograph is presented clearly if not in an orderly manner. with excellent photographs, several charts and curves, and a good bibliography. It is indicated that further experiments are in progress which will meet certain problems, especially those introduced in the drying of sodium penicillin. A method of vacuum spin-freezing is being developed which will permit the loading of the drying apparatus with liquid material, thus rendering unnecessary all prefreezing and low temperature storage.

TUMORES Y SEUDOTUMORES DE LA MAMA. ESTUDIO DE INVESTIGACIÓN EXPERIMENTAL SU PROFILAXIS Y TRATAMIENTO. By Dr. Jacinto Moreno. Pp. 142, with 40 illustrations. Buenos Aires: López & Etchegoyen, 1946.

ANATOMÍA PATOLÓGICA DE LA INFLAMACIONES ESPECÍFICAS. Por el Doctor Ramiro Pico Duni, docente libre de la cátedra de anatomía y fisiología patológicas de la Facultad de Ciencias Médicas de Buenos Aires, Jefe del laboratorio de anatomía patológica del Hospital Argerich. Pp. 189, with 62 illustrations. Buenos Aires: López & Etchegoyen, 1946.

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Subject entries are made for all articles. Author entries are made for original articles. Book Reviews and Obituaries are indexed under these headings in alphabetical order under the letters B and O, respectively.

- Abdomen:** See Gastrointestinal Tract; etc.
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